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RESPONSES OF DORMANT CUTTINGS OF *LONICERA TARTARICA* TO SOLUTIONS OF INDOLYLACETIC ACID AND NUTRIENT SALTS[1]

BY N. H. GRACE[2] AND M. W. THISTLE[3]

Abstract

Cuttings of dormant *Lonicera tartarica*, collected in March, were treated with a factorial series of indolylacetic acid and nutrient solutions. Indolylacetic acid was used at dosages of 0, 10, 50, and 100 p.p.m. in conjunction with 0, 1, and 10 concentrations of a modified Hoagland's nutrient solution. Indolylacetic acid treatment significantly increased the percentage of rooting, and the number and total length of roots, the fresh root weight and the green weight of leaf per group of cuttings treated, the higher concentrations having the greater effect. The use of nutrient also significantly affected each of the foregoing characters. The results suggest that some dormant cuttings may be deficient in minerals essential for rooting, and that there is an optimum nutrient concentration somewhere below the highest used in this experiment.

Recent communications describe the effects of treatment of dormant *Lonicera tartarica* cuttings with dusts containing indolylacetic acid, cane sugar and ethyl mercuric phosphate; and solutions of indolylacetic acid and cane sugar (3, 4). The present communication describes an experiment in which *Lonicera tartarica* cuttings, from the same collection of material, were treated with a series of indolylacetic acid and nutrient solutions. The experimental arrangement also permits a study of the interaction between the growth-stimulating chemical, indolyl-3-acetic acid, and a mixture of nutrient salts. This is of interest, since little has been reported on these interaction effects on plant cuttings, though it is known that their responses are affected by certain nutrient treatments (2).

Experimental

The experiment was of factorial design, with four concentrations of indolylacetic acid and three of a mixture of nutrient salts. Indolylacetic acid was used at 0, 10, 50, and 100 p.p.m. in solution. The nutrient solution was used at levels of 0, 1 nutrient, which contained in p.p.m., K 235, Ca 200, Mg 49, PO_4 95, NO_3 940, SO_4 192, Na 47, Cl 73, B 1, Mn 0.44 and Zn 0.16; and 10 nutrient, which contained 10 times these amounts. This required a series of 12 treatment combinations, which were planted in four randomized blocks.

[1] *Manuscript received August 17, 1939.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. N.R.C. No. 842.

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The entire experiment of four blocks of 12 treatments required 288 cuttings.

Stock solutions of indolylacetic acid and the mixture of nutrient salts were prepared (4). Each contained twice the amount of material required to give the highest treatment. These two solutions, by dilution and mixing, enabled the ready preparation of each individual concentration and mixture required. All the solutions were made with distilled water.

The cuttings were dormant material, 1938 wood, and were about 12 in. long. They were treated in groups of 24 cuttings (the four replicates) with 100 cc. of solution in 250-cc. beakers. The cuttings were held in solution at the laboratory temperature of approximately 72° F. for a treatment period of 24 hr. They were then rinsed and planted immediately in brown sand in a propagation frame equipped with bottom heat cables. The sand was maintained at about 72° F., while the room temperature dropped to 65° F. during the night and rose to around 75° F. during the day. However, during the last two weeks of the rooting period, day temperature frequently went up over 80° F. Further, this room had considerably greater light intensity than the one in which the other two series of *Lonicera* treatments were propagated (3, 4).

The cuttings were planted April 1 and removed for measurements May 9, 1939. A record was made of the number of cuttings rooted, the number and total length of roots, the fresh root weight, and the green weight of leaf for each group of six cuttings. In this experiment the use of 6 instead of 10 cuttings to a group, and rather indifferent rooting of the controls, made it desirable to make comparisons by groups rather than in terms of the individual rooted cutting. All the data were subjected to analyses of variance.

Results

The material dealt with in the present experiment proved to be highly variable, and much of the data appeared to be non-normal. Skewed data being unsuitable for the application of the analysis of variance, various schemes of transformation of the raw data were resorted to (1). The data on the number of cuttings rooted were treated by means of the inverse sine transformation, total root length data were transformed logarithmically, and the square root transformation was used on the data for the number of roots formed. The data on the weights of leaves and roots approximated normality, and were treated as they stood. For the purpose of direct comparison, treatment means of the untransformed data are presented in Table III.

It will be observed from Table I that there were significant treatment differences in respect of all five characters dealt with. Separation of the treatment components would seem to demonstrate the undoubted physiological activity of the nutrient solution employed, as well as a marked response to the use of indolylacetic acid. No significant interaction could be demonstrated, *i.e.*, there was no differential response at different nutrient levels to the same hormone dosage.

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF *Lonicera tartarica* TO HORMONE AND NUTRIENT SOLUTIONS

Source of variance	Degrees of freedom	Mean square				
		Number of cuttings rooted (transformed data)	Total root length (transformed data)	Number of roots (transformed data)	Green weight of leaves	Fresh weight of roots
Blocks	3	346.26	.88	17.91	2.08	.465
Treatments:	11	1809.19***	5.82***	55.26***	4.56***	1.354***
Indolylacetic	3	4621.49***	17.48***	152.96***	10.23**	3.361***
Nutrient	2	1837.09**	4.00†	39.87**	4.98†	1.044*
Interaction indolyl × nutrient	6	393.73	.61	11.55	1.59	.453
Error	33	265.38	1.29	6.48	1.55	.247

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
† *Mean square approaches significance. The two nutrient levels were thrown together, giving a comparison between nutrient and no nutrient. Resulting mean square exceeds 5% level of significance on remaining degree of freedom.*

Table II shows the effect of hormone and nutrient preparations on the number of cuttings rooted, total root length, number of roots, green weight of leaves, and fresh weight of roots per group.

In general, a significant increase is to be noted in all five criteria owing to the use of indolylacetic acid, the most pronounced effect being secured from 50 to 100 p.p.m. However, even the lowest concentration, 10 p.p.m., had

TABLE II

RESPONSE OF *Lonicera tartarica* TO HORMONE AND NUTRIENT SOLUTIONS

Treatment	Number of cuttings rooted (transformed data)	Total root length (transformed data)	Number of roots (transformed data)	Green weight of leaves, gm.	Fresh weight of roots, gm.
0 p.p.m. Indolylacetic	3	0.2	0.3	0.7	0.02
10 p.p.m. Indolylacetic	20*	1.7*	2.0	1.7*	0.20
50 p.p.m. Indolylacetic	41*	2.7*	6.2*	3.0*	1.14*
100 p.p.m. Indolylacetic	45*	2.8*	7.9*	1.9*	0.85*
Necessary difference for 5% level of significance	14	.9	2.1	1.0	0.41
0 Nutrient	16	1.3	3.0	1.2	0.30
1 Nutrient	37*	2.3	5.9*	2.3	0.81*
10 Nutrient	28*	1.8	3.4	2.0	0.55
Necessary difference for 5% level of significance	12	0.7	1.8	0.8	0.36

* *Exceeds control 5% level of significance.*

significant effects on the number of cuttings rooted, total root length, and green weight of leaves, although not on the number of roots and fresh weight of roots. The 50 and 100 p.p.m. means did not suffer significantly between themselves, except in respect of leaf weight, in which case the 50 p.p.m. mean was significantly higher than the 100 p.p.m. mean

In all cases the use of nutrient solution also produced a significant stimulation. Of the two levels of nutrient employed, the evidence favours the lower, and the existence of an optimum concentration is strongly suggested.

TABLE III

RESPONSE OF *Lonicera tartarica* TO HORMONE AND NUTRIENT SOLUTIONS
Treatment means of untransformed data

Treatment	Per cent of cuttings rooted	Total root length, mm.	Number of roots
0 p.p.m. Indolylacetic	3	27	1
10 p.p.m. Indolylacetic	17	327	7
50 p.p.m. Indolylacetic	47	1951	54
100 p.p.m. Indolylacetic	50	1844	79
0 Nutrient	16	616	24
1 Nutrient	42	1787	60
10 Nutrient	30	709	22

Certain general comparisons may be made with the results of dust and solution treatments of the same dormant material, though the higher temperature and greater light intensity in which the cuttings of this experiment were propagated render it necessary to exercise care in making comparisons (3, 4). It seems obvious, however, that this experiment has been carried out under sub-optimum conditions. Either the higher temperature of the room, or the greater light intensity, or both these factors, had adverse effects on the responses. Although these comparisons seem to indicate that nutrient salts had greater effects than sugar on the rooting responses of cuttings of dormant *Lonicera tartarica*, further experiments under identical conditions will be required before general conclusions can be reached.

Acknowledgment

The writers wish to acknowledge their indebtedness to Dr. J. W. Hopkins for his assistance in planning the statistical arrangement of the experiment.

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EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID IN TALC ON THE ROOTING OF CUTTINGS[1]

By N. H. Grace[2]

Abstract

Cuttings of two herbaceous and two dormant woody plants were treated with a factorial series of talc dusts containing cane sugar, ethyl mercuric phosphate and indolylacetic acid. The effect of the dusts on cuttings of *Coleus Blumei* and *Iresine Lindeni* was determined by the number of roots per rooted cutting, the length of root mass and dry weight of roots. Each of the three factors gave at least one significant effect with both plants. *Iresine Lindeni* cuttings showed two significant interactions, one between organic mercury and indolylacetic acid on the number of roots per rooted cutting, the other between sugar and indolylacetic acid on the length of root mass. Dormant *Lonicera tartarica* cuttings showed significant effects from indolylacetic acid on the number of cuttings rooted, the number and length of roots per rooted cutting, the mean root length, and fresh root weights. Green leaf weights of this plant were significantly affected by sugar, and the sugar × organic mercury, and sugar × organic mercury × indolylacetic acid interactions. Fresh root weights also gave a significant triple interaction. The number of *Physocarpus opulifolius* cuttings rooted was significantly increased by organic mercury as were the dry root weights. Root weights also were affected by sugar treatment. This plant failed to make any significant response to indolylacetic acid treatment.

The results indicate that cane sugar and ethyl mercuric phosphate, as well as indolylacetic acid, affect some of the rooting responses of plant cuttings. It is suggested that the dust method of treating cuttings may be used to supply factors, other than the recognized growth stimulating chemicals, that are advantageous to successful vegetative propagation of plants.

The carrier dust method of applying root growth promoting chemicals to plant cuttings has been shown to be both effective and convenient (6). Preliminary work on the use of hormone dusts as a vehicle for nutrient salts indicated that improved rooting could be effected by this means. While numerous factors are known to influence the response of plants to growth stimulating chemicals, considerable work has been done on the effects of sugars and auxins (1–3, 5, 8, 10, 12–15, 17, 18). In consequence, detailed study has been directed first to hormone dust mixtures containing sugar. The effects of nutrient salts are under consideration also and will be reported later. The present communication describes the results of experiments in which cuttings were treated with a series of talc dusts containing indolyl-3-acetic acid, cane sugar, and an organic mercurial disinfectant, ethyl mercuric phosphate. Although organic mercurials are used extensively in seed disinfection, little is known as to their effect on the rooting responses of cuttings. The organic mercurial disinfectant was used in an effort to provide against the possibility of infections from the use of dusts containing large amounts of sugar.

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Experimental

The effects of the three chemicals, cane sugar, ethyl mercuric phosphate, and indolylacetic acid, were investigated by an experiment of factorial design. The series of dust mixtures comprised cane sugar at five concentrations, namely, 0, 1, 5, 10, and 20%, in combination with ethyl mercuric phosphate at 0, 10, 50, and 100 p.p.m., and indolylacetic acid at 0 and 1000 p.p.m. The complete series of possible dosage combination of the three chemicals required the preparation of 40 different dusts. There were three replicates of the 40 individual treatments and one level of precision for all the different comparisons. This factorial arrangement is highly efficient in that it enables the use of the entire 120 groups of cuttings in determining the effect of each chemical or interaction.

The dust mixtures were prepared in talc by the grinding mix method (6). Firstly, dusts containing 10,000 p.p.m. indolylacetic acid and 1000 p.p.m. ethyl mercuric phosphate were prepared. These were then diluted with talc, and cane sugar was added directly to give the various concentrations and mixtures required. The source of the ethyl mercuric phosphate was a commercial seed disinfectant that contains 5% of the salt by weight. Analysis of the mercurial disinfectant disclosed that the P_2O_5 content was approximately 1.0%.* This means that dusts containing 10, 50, and 100 p.p.m. of ethyl mercuric phosphate carry approximately 2, 10, and 20 p.p.m. of P_2O_5 respectively. Preliminary experiments with the organic mercurial in talc indicated that 1000 p.p.m. had a markedly damaging effect on several varieties of plant cuttings; this damage was reduced but apparent at 500 p.p.m.; however, injury could not be detected visually when 100 p.p.m. or less was used.

Four varieties of plants were used in the investigation. Cuttings of two herbaceous plants, *Iresine Lindeni* Lem. and *Coleus Blumei* Benth., provided data on the effect of treatments on the number, mass length, and weight of roots. Since rooting was almost 100% for all treatments, these plants did not give information on the initiation of roots. Cuttings of *Iresine Lindeni*, 10 cuttings to a group, were treated and planted August 17, 1938, in an outdoor propagation frame containing brown sand, and covered with a factory cotton screen. The cuttings were taken up for measurements September 9, 1938. *Coleus Blumei* cuttings, 5 to a group, were planted in an identical frame August 20, 1938 and removed September 14, 1938. Two species of dormant woody cuttings were placed after treatment in an indoor propagation frame containing brown sand and equipped with bottom heat. The sand temperature was thus maintained at about 72° F., while that of the room was about 65° F. Both varieties had 10 cuttings in a group, and observations were made of the effect of treatments on the rooting of the cuttings, as well as effects on number and length of roots. *Physocarpus opulifolius* Maxim.

* *The analysis was carried out by Mr. C. W. Davis, Division of Chemistry, National Research Laboratories.*

cuttings* were treated October 20, 1938 and removed for measurements December 14, 1938. *Lonicera tartarica* L. cuttings were treated March 30, 1939 and removed May 1, 1939.

Freshly prepared cuttings were dipped in dust in groups of 10 cuttings (5 in the experiment with *Coleus*). The group was given a few quick turns in the dust, withdrawn, and shaken to remove excess. Treated cuttings carried a fairly uniform layer of dust over the cut ends and up the stem from the base for a distance of about 1 in. They were planted in fairly wide trenches in sand to avoid rubbing off appreciable amounts of the adhering dust preparations. The dusts containing 20% of cane sugar had a tendency to cake. However, the bottle was shaken and, if necessary, the dust poured out on paper and any cakes were pulverized with a spatula. Changes in dust consistency become more marked above the 20% level, and for this reason that concentration of sugar was chosen as the maximum.

The number of roots was counted on each rooted herbaceous cutting and expressed as the number of roots per rooted cutting for each group. One measurement was made of the length of the root mass of each cutting and expressed as mean length of root mass per group. While individual root lengths are to be preferred, the large number of roots rendered such measurements impossible in a reasonable time. Finally, dry root weights were determined for each group of cuttings. This was done by determining the difference in the weight of washed roots dried at 95° C., before and after ashing.

The number of dormant cuttings rooted per group was recorded and counts were made of the number of roots on each cutting. Total root length per rooted cutting was measured and mean root lengths calculated. Root weights were also obtained, dry weights for the roots of *Physocarpus opulifolius* and fresh root weights (7) for *Lonicera tartarica*. In addition, the weight of green leaf produced by each group of *Lonicera* cuttings was determined.

All the data for both herbaceous and dormant woody cuttings were subjected to analyses of variance.

Results

The results are presented in two parts; the first deals with the responses of herbaceous cuttings, the second with those of dormant woody material.

Responses of Herbaceous Cuttings

The results of analyses of variance of the data secured are given in Table I. It is apparent that indolylacetic acid had highly significant effects on the number of roots per rooted cutting and the dry weight of roots of both plants, but significantly affected the length of root mass of *Iresine* only. Sugar and organic mercury showed significant effects in at least one species on each of the three responses studied.

* *The prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF HERBACEOUS CUTTINGS TO TALC DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean Square					
		Number of roots per cutting rooted		Length of root mass, mm.		Dry weight of roots, gm. × 1000	
		Iresine	Coleus	Iresine	Coleus	Iresine	Coleus
Blocks	2	115.64*	99.56	118.86*	1182.21***	4.795***	23.109**
Indolylacetic acid dosage	1	2020.48***	4040.28***	3525.17***	61.20	6.873***	40.095***
Sugar dosage	4	88.27*	169.05*	26.38	344.11**	0.537	7.078*
Organic mercury dosage	3	94.20*	29.20	29.50	247.13*	0.522	0.940
Interaction Sugar × organic mercury dosage	12	31.40	35.63	21.24	60.66	0.181	1.669
Interaction Sugar × indolylacetic acid dosage	4	10.64	54.86	73.99*	66.67	1.008	2.191
Interaction Organic mercury × indolylacetic acid dosage	3	150.19**	68.19	10.83	59.33	0.348	2.211
Interaction Sugar × organic mercury × indolylacetic acid dosage	12	41.83	79.41	14.15	73.63	0.347	3.039
Error	78	29.45	47.66	25.83	88.82	0.458	2.653

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

Two significant interactions are to be noted, the first between organic mercury and indolylacetic acid dosage on the number of roots per rooted cutting of *Iresine*, and the second between sugar and indolylacetic acid dosage on the length of root mass of the same species. It is also apparent that the number of roots per rooted cutting was the measurement least affected by block variation; this observation has been noted on several occasions.

The treatment averages in Tables II and III indicate the nature of the main effects demonstrated by the analyses of variance. Averaged over all concentrations of sugar and mercury, treatment with 1000 p.p.m. indolylacetic acid increased the number and dry weight of roots of both plants, but decreased the length of root mass of *Iresine*. Sugar treatment increased the number, the length of root mass and weight of roots of *Coleus;* however, it decreased the number of roots per rooted *Iresine* cutting significantly at the 5 and 10% levels. Organic mercury decreased the length of root mass of *Coleus* and increased the number of roots per rooted *Iresine* cutting.

The data in Table IV show the average interaction effects over all sugar dosages of organic mercury and indolylacetic acid on the number of roots per rooted *Iresine* cutting. It is apparent that organic mercury alone failed to affect the number of roots significantly, though a slight reduction is suggested with increasing concentration. Indolylacetic acid alone did not

TABLE II

AVERAGE RESPONSES OF *Coleus Blumei* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

	Effects of treatment with													
	Indolylacetic acid, p.p.m.		Necessary diff., 5% level	Cane sugar, %					Necessary diff., 5% level	Ethyl mercuric phosphate, p.p.m.				Necessary diff., 5% level
	0	1000		0	1	5	10	20		0	10	50	100	
Number of roots per rooted cutting	19.5	31.1*	2.5	21.9	25.0	23.7	28.1*	27.8	3.9					
Length of root mass, mm.				51.9	58.3*	57.1	56.9	62.5*	5.4	60.4	58.6	56.6	53.8*	4.9
Dry weight of roots, gm.	0.072	0.109*	0.019	0.070	0.086	0.083	0.097	0.116*	0.030					

* *Significantly different from the corresponding 0 value.*

TABLE III

RESPONSES OF *Iresine Lindeni* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

	Effects of treatment with													
	Indolylacetic acid, p.p.m.		Necessary diff., 5% level	Cane sugar, %					Necessary diff., 5% level	Ethyl mercuric phosphate, p.p.m.				Necessary diff., 5% level
	0	1000		0	1	5	10	20		0	10	50	100	
Number of roots per rooted cutting	25.5	33.7*	2.0	32.1	29.1	28.3*	27.5*	31.0	3.1	27.7	30.5	31.6*	28.53	2.8
Mean length of root mass, mm.	38.9	28.1	1.9											
Dry weight of roots, gm.	0.031	0.046*	0.004											

* *Significantly different from the corresponding 0 value.*

TABLE IV

AVERAGE RESPONSES OF *Iresine Lindeni* CUTTINGS FROM THE INTERACTION OF INDOLYLACETIC ACID AND ETHYL MERCURIC PHOSPHATE DOSAGE

	Indolyl-acetic acid, p.p.m.	Ethyl mercuric phosphate, p.p.m.			
		0	10	50	100
Number of roots per rooted cutting	0	26.2	27.4	24.9	23.4
	1000	29.3	33.6	38.3	33.6
Necessary difference, 5% level					3.96

increase the number of roots to a significant extent. However, significant increases followed combination with the three organic mercury levels, the maximum response being secured by the use of 50 p.p.m.

Table V shows the average effects over all mercury dosages of indolylacetic acid and cane sugar on the length of the root mass of *Iresine* cuttings. The only significant effect of sugar without indolylacetic acid was a reduction in length at the 20% level over that at the 1% concentration. Indolylacetic acid alone reduced the length markedly: with 20% sugar the length was significantly increased over the value at 10%. The 20% sugar concentration appears to protect the plant by decreasing the tendency to shorten the length of root mass.

TABLE V

AVERAGE RESPONSES OF *Iresine Lindeni* CUTTINGS FROM THE INTERACTION OF INDOLYLACETIC ACID AND CANE SUGAR DOSAGE

	Indolyl-acetic acid, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Length of root mass, mm.	0	40.6	41.0	37.4	39.2	36.8
	1000	28.4	27.9	27.9	25.1	31.1
Necessary difference, 5% level						4.2

RESPONSES OF DORMANT WOODY CUTTINGS

The results of analyses of variance of the responses of dormant woody cuttings to the various talc dusts are given in Table VI. Organic mercury had a highly significant effect on the number of rooted *Physocarpus* cuttings and on the dry root weights. These effects from treatment of cuttings with organic mercury are noteworthy. Sugar treatment had a significant effect on the root length per rooted cutting and on the dry weight of roots of *Physocarpus*. This plant, however, failed to show any significant

TABLE VI

ANALYSIS OF VARIANCE OF RESPONSES OF DORMANT CUTTINGS TO TALC DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square										
		Number of cuttings rooted		Number of roots per rooted cutting		Root length per rooted cutting		Mean root length		Root weights		Green weight of leaves
										Dry	Fresh	
		Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Physocarpus	Lonicera	Lonicera
Blocks	2	0.5799*	88.47	0.2194	3.15	1268	4128	658.2*	272.4	89	0.0711	35.71***
Indolylacetic acid dosage	1	0.0052	4034.48***	0.0210	105.28***	11900	429364***	270.0	2698.0*	2467	12.4936***	3.85
Sugar dosage	4	0.3142	35.78	0.1513	12.49	9247*	18982	115.3	189.1	3348*	0.4541	5.45**
Organic mercury dosage	3	0.9190***	30.34	0.0552	6.74	6722	14118	96.6	548.2	8550***	0.3571	3.64
Interaction Sugar × organic mercury dosage	12	0.1257	159.42	0.0775	6.59	3163	17784	156.9	222.5	1613	0.3963	2.77*
Interaction Sugar × indolylacetic acid dosage	4	0.2094	103.95	0.1431	4.49	2945	19788	141.7	380.9	857	0.4391	1.20
Interaction Organic mercury × indolylacetic acid dosage	3	0.1583	123.86	0.0535	0.67	2891	1893	81.2	680.0	1700	0.2835	1.29
Interaction Sugar × organic mercury × indolylacetic acid dosage	12	0.1575	141.01	0.0470	7.43	1127	24001	62.1	277.8	743	0.5643*	2.67*
Error	78	0.1474	95.46	0.0789	6.73	3578	14408	132.1	403.6	1307	0.2414	1.36

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VII

AVERAGE RESPONSES OF *Physocarpus opulifolius* CUTTINGS TREATED WITH DUSTS CONTAINING CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID

	Effects of treatment with										
	Ethyl mercuric phosphate, p.p.m.				Necessary diff., 5% level	Cane sugar, %					Necessary diff., 5% level
	0	10	50	100		0	1	5	10	20	
Number of cuttings rooted—											
Transformed data†	2.28	2.61*	2.58*	2.68*	0.207						
Percentage rooting	50	65	63	69							
Root length per rooted cutting, mm.						183	176	221*	187	213	34
Dry root weight, mg.	67	90*	94*	108*	18	82	73	96	96	102	20

* *Significantly different from corresponding 0 value.*

† *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (4).*

response to indolylacetic acid treatment. *Lonicera*, on the other hand, showed significant responses to indolylacetic acid, the green weight of leaves produced being the sole exception; furthermore, in only one instance did the level of significance fail to attain the 0.1% point. Sugar had a significant effect on the weight of green leaf; this response, also, indicates significant interactions between sugar and organic mercury and sugar, organic mercury, and indolylacetic acid. Fresh root weights bring out another significant triple interaction. On the whole, the two experiments fail to show very significant block effects. However, the green weight of leaf of *Lonicera* cuttings shows a very highly significant block variation. This block effect appears to have been largely due to differences in light; the blocks receiving the greater intensity of light showed substantially more leaf production.

The significant responses of *Physocarpus* cuttings are given in Table VII. It is apparent that organic mercury increased the number of cuttings rooted at all three concentrations, which do not differ among themselves. On the average, marked stimulation of dry root weight resulted from organic mercury treatment, the 100 p.p.m. level being significantly above the 10, although not above the 50. Average dry root weights at 5, 10, and 20% of sugar were significantly better than that at 1% sugar, but were not significantly greater than the value at zero sugar. Sugar at the 5% level significantly increased the length of root per rooted cutting.

Average effects over all dosages of sugar and mercury of indolylacetic acid on *Lonicera* cuttings are given in Table VIII. Indolylacetic acid increased the percentage of cuttings rooted, the number and length of roots per rooted cutting, the mean root length and the fresh root weight. The substantial increase in mean root length is of interest, as solution treatment usually reduces the length if the effect is significant.

TABLE VIII

AVERAGE RESPONSES OF *Lonicera tartarica* CUTTINGS TO DUST TREATMENT WITH INDOLYLACETIC ACID

Data are means of 60 groups of 10 cuttings

Indolylacetic acid, p.p.m.	Number of cuttings rooted		Number of roots per rooted cutting	Root length per rooted cutting, mm.	Mean root length, mm.	Fresh root weight, gm.
	Trans-† formed data	Per cent				
0	35.21	34	3.4	127	37.6	0.345
1000	46.81	53	5.3	247	47.1	0.990

† *Data transformed to angles (4).*

In Table IX are shown the average effects of sugar treatment over both indolylacetic acid dosages, and the sugar × organic mercury interaction on the green weight of leaf of *Lonicera*. The 1% sugar mean does not differ

TABLE IX

AVERAGE GREEN WEIGHT OF LEAVES OF *Lonicera tartarica* CUTTINGS ON DUST TREATMENT WITH CANE SUGAR AND ETHYL MERCURIC PHOSPHATE

Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
	0	1	5	10	20
0	8.10	8.17	8.02	6.12	7.25
10	8.29	7.60	6.22	6.27	7.57
50	8.52	8.40	8.27	7.22	7.38
100	8.23	7.55	7.18	8.65	7.67
Means for sugar treatments	8.28	7.93	7.42	7.06	7.47

Necessary difference, 5% level, interaction 1.34, treatment means 0.67

significantly from the control. The 5, 10, and 20% sugar means are all significantly below the control, but do not differ significantly among themselves. The 10% mean also is significantly below the 1% mean. The sugar × organic mercury interaction appears to be such that at the 0, 1, and 20% sugar levels, mercury had no significant effect. At the 5 and 10% sugar levels, on the other hand, mercury had a detrimental effect at the 10 p.p.m., and at the 0 and 10 p.p.m. concentrations respectively.

In Table X are given the average effects of sugar and organic mercury on the response of leaf weights of groups of *Lonicera* cuttings to indolylacetic acid treatment. The leaf weights are given in the form of differences between values obtained with and without the use of indolylacetic acid, permitting examination of the triple interaction. At 0 and 5% sugar levels, the addition of 10 p.p.m. organic mercury produced significant reduction in response to indolylacetic acid, while at the 10% sugar level 100 p.p.m. organic mercury is necessary to produce injurious effects. However, the 20% sugar level has significantly detrimental effects at zero organic mercury, and the addition of organic mercury reduced the effect to insignificance.

TABLE X

INTERACTION OF EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID ON THE WEIGHT OF LEAVES PRODUCED BY *Lonicera tartarica* CUTTINGS

	Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Differences in leaf weight, gm. (Differences between groups receiving and not receiving indolylacetic acid)	0	0.40	0.23	0.78	0.08	−1.38
	10	−0.98	0.43	−0.78	0.33	0.07
	50	−0.45	0.30	0.13	−0.22	−0.22
	100	0.13	−0.65	−0.08	−1.35	−0.37

Necessary difference, 5% level, 1.34

The average effects of sugar and organic mercury on the response of fresh root weights are given in Table XI. The fresh root weights are given in the form of differences between values obtained with and without the use of indolylacetic acid, permitting examination of the triple interaction on the fresh root weight of *Lonicera*. It appears that in the absence of mercury, indolylacetic acid has a significant effect at the 5% sugar level. With 5% sugar the addition of 10 p.p.m. organic mercury reduces the effect to insignificance, but the effect reaches significance again at the 100 p.p.m. concentration of organic mercury. With 10% sugar the addition of 10 p.p.m. organic mercury does not reduce the effect, but this does occur at the 100 p.p.m. organic mercury concentration. In other words the organic mercury effect reverses from 5 to 10% sugar levels.

TABLE XI

INTERACTION EFFECTS OF CANE SUGAR, ETHYL MERCURIC PHOSPHATE, AND INDOLYLACETIC ACID ON THE FRESH ROOT WEIGHT OF CUTTINGS OF *Lonicera tartarica*

	Ethyl mercuric phosphate, p.p.m.	Cane sugar, %				
		0	1	5	10	20
Differences in fresh root weights, gm. (Differences between groups receiving and not receiving indolylacetic acid)	0	0.13	0.16	0.81	0.58	0.11
	10	0.23	0.42	−0.14	0.81	0.21
	50	0.42	0.58	0.43	0.51	0.21
	100	0.34	0.11	0.70	−0.13	−0.05

Necessary difference, 5% level, 0.56.

A factorial series of dusts containing cane sugar, ethyl mercuric phosphate, and indolylacetic acid has also been applied to dormant cuttings of Norway spruce. Significant effects from all three factors were obtained on the length of root per rooted cutting and the interactions between sugar and indolylacetic acid, and sugar and organic mercury also were significant. These results will be given in detail shortly in a publication dealing with the vegetative propagation of conifers.

Conclusions

Each of the three chemicals, cane sugar, ethyl mercuric phosphate, and indolylacetic acid, had a significant effect on at least one of the responses of both herbaceous plants studied. Significant interactions of organic mercury and indolylacetic acid and sugar and indolylacetic acid were shown by *Iresine* cuttings. However, the response of both plants was not always the same to a given chemical. For instance, sugar treatment increased the number of roots on *Coleus* cuttings but decreased the number on *Iresine* cuttings. It must be pointed out that physiological activity has been determined by the

responses of cuttings that actually rooted, and it may not be inferred that all three chemicals will be effective in the treatment of herbaceous cuttings that root with difficulty. It seems reasonable, however, to expect that treatments which have a beneficial effect on the number of roots, the length of root mass, and the root weight will be of value in promoting the successful propagation of healthy vigorous plants from cuttings.

The dormant woody cuttings also showed significant effects from the three chemicals. Very highly significant effects from indolylacetic acid are shown by cuttings of *Lonicera*. The green leaf weights of this plant indicated significant sugar effects and an interaction between sugar and organic mercury and an interesting triple interaction; the latter was also shown by the fresh root weights. *Physocarpus* failed to respond significantly to indolylacetic acid in every case, but gave two highly significant effects from organic mercury and two less significant effects from sugar. The results from the two varieties of dormant woody cuttings consequently also suggest a measure of physiological activity for each of the three substances studied. The rather slight positive and detrimental effects from sugar suggest that dormant woody cuttings have an adequate reserve of carbohydrate material.

One of the most interesting features of the results is the physiological activity of organic mercury. Conflicting claims have been made for organic mercurials as stimulants to germination and the early growth of seedlings (9, 16). These results for the responses of cuttings certainly suggest physiological activity rather than mere fungicidal effects, though the latter may be a factor when sugar is present. However, it must be pointed out that a small but appreciable amount of phosphate is present along with the organic mercury. While it is unlikely that the marked activity of 10 p.p.m. ethyl mercuric phosphate can be attributed solely to the 2 p.p.m. P_2O_5 present, this possibility must be considered. Experiments now under way involve the use of organic mercurial preparations free from phosphate.

The results, as a whole, suggest that the dust method of treating cuttings may be used to provide accessory factors along with recognized growth stimulating chemicals such as indolylacetic or naphthylacetic acids. This conclusion is borne out by the results of Stoutemeyer, who has effected improved rooting through the addition of thiourea to treating dusts (11). Before optimum combinations and concentrations can be established, extended experimentation will be required with cuttings from a wide variety of plants, and with carbohydrates, nutrient salts, and other possible factors.

Acknowledgments

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RESPONSES OF DORMANT CUTTINGS OF *LONICERA TARTARICA* TO SOLUTIONS OF CANE SUGAR AND INDOLYLACETIC ACID[1]

By N. H. Grace[2]

Abstract

Cuttings of dormant *Lonicera tartarica*, collected in March, were treated with a factorial series of indolylacetic acid and cane sugar solutions. Indolylacetic acid was used at concentrations of 0, 10, 50, and 100 p.p.m., while cane sugar was present at 0, 1, 5, and 10%. Indolylacetic acid treatment greatly increased the percentage of cuttings rooted, the number and length of roots per rooted cutting, the fresh root weight and the green weight of leaf produced. Cane sugar treatment alone or in combination with indolylacetic acid failed to show any significant effects, suggesting that dormant cuttings of this plant have an adequate reserve of carbohydrate material.

Apart from a somewhat greater effect of treatment on the percentage of rooting, the results are in essential agreement with those previously secured from dormant October cuttings. In comparison with a parallel experiment on the dusting of March cuttings propagated in the same frame, solution treatment had the greater effect on all the responses considered except green weight of leaf produced, which was greater following dusting.

A recent communication describes the effects of treating *Lonicera tartarica* cuttings with dusts containing cane sugar, ethyl mercuric phosphate, and indolylacetic acid (2). Since there are some differences in the response of plant cuttings to the dust and solution methods of treatment, it was considered of interest to carry out an experiment in which cane sugar and indolylacetic acid were applied by solutions. The present communication describes an experiment that investigates the effects of both these factors and their interactions, using the solution method of applying the chemicals to the cuttings. The cuttings were from the same collection of material and propagated in the same frame as in the series of dust treatments mentioned (2). This fact permits a rough comparison of the responses of cuttings of this plant to the dust and solution methods of applying indolylacetic acid.

The responses of this March collection of *Lonicera tartarica* cuttings also are compared with those obtained with an October collection and treated with the same concentrations of indolylacetic acid (3).

Experimental

The experiment was of factorial design; cane sugar and indolylacetic acid were used at four concentrations, namely, 0, 1, 5, and 10% of the former, and 0, 10, 50, and 100 p.p.m. of the latter in solution. Indolylacetic acid solution was made by dissolving 0.2000 gm. of the pure chemical in 2 cc. of 95% alcohol and making up to one litre with distilled water, giving a 200 p.p.m. solution. A solution of 20% cane sugar was prepared by dissolving the commercial product in water. These two solutions, by dilution and mixing,

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enabled the ready preparation of each individual concentration and mixture required. All the solutions were made with distilled water.

Each of the 16 possible combinations of indolylacetic acid and sugar was applied to four replicate groups of 10 cuttings. The cuttings (640 in all) were dormant material, 1938 wood, and were about 12 in. long. They were treated in groups of 40 cuttings (the four replicates) with 150 cc. of solution in 400-cc. beakers. The cuttings were held in the laboratory at approximately 72° F. for the treatment period of 24 hr. They were then rinsed and planted immediately in brown sand in a propagation frame equipped with bottom heat cables. The planting arrangement was in the form of four randomized blocks, each containing one replicate (10 cuttings) of each of the 16 treatments. The sand temperature was maintained at 72° F., while the room temperature ranged close to 65° F. The large dust series of *Lonicera* cuttings mentioned was present in the other end of the same propagation frame.

The cuttings were planted March 31, 1939, and removed for measurement May 5. Record was made of the number of cuttings rooted in each group, the number and length of roots per rooted cutting, the mean root length, fresh root weights, and the weight of green leaf produced. All the data were subjected to analyses of variance.

Results

In Table I are given results of the analyses of variance of the several observations made on the rooted cuttings. Highly significant effects from indolylacetic acid were shown by five of the six responses examined; the mean root length was not affected. Cane sugar treatments alone, or in interaction with indolylacetic acid, failed to show any significant effects. Furthermore,

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS TO SOLUTIONS OF CANE SUGAR AND INDOLYLACETIC ACID

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings rooted	Number of roots per rooted cutting	Length of roots per rooted cutting	Mean root length	Fresh root weight	Green weight of leaves
Blocks	3	21.20	10.23	63565	464.43**	0.8500	4.703
Cane sugar dosage	3	26.04	19.01	11588	31.60	0.0689	1.115
Indolylacetic acid dosage	3	5101.05***	944.43***	971896***	158.68	46.6713***	41.265***
Interaction cane sugar × indolylacetic acid dosage	9	255.69	19.55	38502	149.95	0.8857	2.562
Error	45	132.74	19.01	29194	98.76	1.2548	2.570

† *Data transformed for analysis of variance, angular method (1).*

** *Exceeds mean square error, 1% level of significance.*

*** *Exceeds mean square error, 0.1% level of significance.*

block variations are negligible except in the case of mean root length, in which they attain the 1% level of significance.

In Table II are given data (averages for all sugar dosages) for the significant responses of *Lonicera* cuttings to indolylacetic acid. The 10 p.p.m. mean is significantly above the 0 value, indicating an increase in the number of cuttings rooted. The 50 and 100 p.p.m. concentration means are significantly above both the 10 and 0 p.p.m. values, but do not differ significantly between themselves.

The 0 and 10 p.p.m. concentration means do not differ in their effect on the number of roots per rooted cutting. The 50 p.p.m. mean is significantly above both the 0 and 10 p.p.m. means but below the value at 100 p.p.m., which affects the number of roots per rooted cutting most markedly. Treatment effects are in the same order by concentration, for the length of root per rooted cutting, the 100 p.p.m. treatment giving significantly more length of roots than any other.

TABLE II

AVERAGE RESPONSE OF DORMANT *Lonicera tartarica* CUTTINGS TO SOLUTIONS OF INDOLYLACETIC ACID

Data are means of 16 groups of 10 cuttings

	Indolylacetic acid solution, p.p.m.				Necessary difference, 5% level
	0	10	50	100	
Number of cuttings rooted of 10 planted					
Transformed data†	37.6	48.8	74.1	72.0	8.2
Per cent rooted	38.1	56.3	87.5	88.8	
Number of roots per rooted cutting	5.1	5.0	13.1	21.1	3.1
Length of roots per rooted cutting, mm.	203	184	527	684	122
Fresh root weight, gm.	0.46	0.71	3.19	3.82	0.80
Green weight of leaves, gm.	5.37	5.61	8.81	7.31	1.14

† *Data transformed for analysis of variance, angular method (1).*

While the 100 and 50 p.p.m. concentrations both significantly increased the fresh weight of root, the values do not differ between themselves. There is no significant difference in the root weight at the 0 and 10 p.p.m. levels. In like manner the 50 and 100 p.p.m. treatments increased the green weight of leaf without differing between themselves, and the 0 and 10 p.p.m. concentrations do not differ.

The absence of significant effects from cane sugar on solution treatment of *Lonicera* cuttings suggests that some varieties of dormant cuttings have adequate reserves of carbohydrate material. It is of interest to point out that dust sugar treatments gave some significant effects, chiefly damaging in nature (2), as statistically significant effects were obtained by this method of treatment.

Comparison of Responses of October and March Collections of *Lonicera tartarica* Cuttings

In Table III are given treatment averages expressed as an average of the control for the responses of October and March collections of dormant *Lonicera* cuttings to solution treatment with indolylacetic acid (3). This method of presenting the data reduces the various responses to a common level, and permits ready comparison of the effects of treatment. Treatment effects on the number and length of roots per rooted cutting and the mean root length are essentially similar for both October and March collections of dormant material. Treatments had a somewhat greater effect on number of cuttings rooted for the March collection. However, this fact is due largely to the greater rooting of the October controls, 59% against 38% for the March, a fact which may be due to the presence of 100 p.p.m. K_2HPO_4 rather than differences in the material at the two dates (3).

TABLE III

Comparison of responses of October and March collections of dormant *Lonicera tartarica* cuttings to indolylacetic acid solutions

Treatment averages expressed as average of control

Month of collection	Indolylacetic acid solution concentration, p.p.m.	Cuttings rooted	Per rooted cutting		Mean root length	Fresh weight of roots	Green weight of leaves
			Number of roots	Length of roots			
October	10	1.16	1.01	0.90	0.84		
	50	1.42	2.97	2.45	0.81		
	100	1.59	4.25	3.29	0.80		
March	10	1.48	0.98	0.91	0.88	1.55	1.04
	50	2.30	2.57	2.60	0.99	6.92	1.64
	100	2.33	4.14	3.37	0.85	8.29	1.36

It is apparent from the data on number of cuttings rooted, for both collections, that there was slight difference between 50 and 100 p.p.m., indicating a levelling-off of effect. This tendency is not shown by the effect of treatment on number or length of roots per rooted cutting; with these responses the effects were markedly greater at 100 p.p.m. than at the 50 p.p.m. level. It is indicated that number and lengths of root per rooted cutting continued to increase with indolylacetic acid concentration even though the effect on rooting approached a constant level. It also is evident that a substantial increase in rooting can occur without marked effects on the number or length of roots. The weight of green leaf produced showed a maximum effect at 50 p.p.m.

Comparison of Effects of Solution and Dust Method of Treatment on Responses of Dormant *Lonicera tartarica* Cuttings

Comparison of the results of this experiment with one in which *Lonicera* cuttings from the same collection were treated with dust preparations, indicates somewhat greater effectiveness for the solution method of applying

indolylacetic acid to this plant (2). It may be seen that 1000 p.p.m. of indolylacetic acid in talc, over all dosages of sugar and organic mercury, effected 53% rooting, while 10 p.p.m. in solution rooted 56% of the cuttings. Solution treatment had the greater effect on all the responses excepting the green weight of leaf produced, which was 7.5 gm. per group of 10 cuttings on 1000 p.p.m. dust treatment, and 7.2 gm. on solution treatment, a mean of the values at the three solution dosages. The results suggest that 1000 p.p.m. indolylacetic acid in talc (used nine months after preparation) had about the same effect as a 10 p.p.m. solution treatment. A somewhat higher concentration of indolylacetic acid in talc would therefore appear to be required to give the rooting effected by solutions on this plant. The relative effectiveness of the dust and solution methods of treating cuttings with growth stimulating chemicals would appear to vary with different plants, since *Ribes odoratum* showed dusts of indolylbutyric acid somewhat more effective than solutions (4).

Acknowledgments

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BUD DEVELOPMENT FOR THE FRUIT-BEARING SPUR OF THE WAGENER APPLE[1]

By Hugh P. Bell[2] and Jean W. McLellan[3]

Abstract

The complete development of the growing tip of the lateral fruit spur of the Wagener apple, from the time of its initiation until it produces mature fruit, requires four seasons. These are referred to as years I, II, III, and IV. The development during years I, II, and III may be divided into six typical phases. The growth during each of these phases is characteristic and different from that of any other stage. The phase just before initiation of the flower extends through June and the first half of July of year III ("off"). During this period the crown broadens and flattens, the pro-meristematic tissue becomes shallow, the scale and leaf primordium bases remain level with the crown and the pro-vascular strands and pith become broadly hemispherical. This phase is followed by flower formation, which is initiated during the last part of July, by the triangular, horizontal, upper surface of the crown becoming circular and developing five sepal primordia for the terminal flower. The flower cluster as a whole is "determinate", but its lateral flowers are axillary in origin and appear in acropetal succession. It is suggested that the changes occurring in the tip of the "off" spur during June, namely, the broadening and flattening of the crown, etc., may be an indication that physiological differentiation of the crown into flower-forming tissue is taking place.

Introduction

The investigation reported below was undertaken at the request of Mr. J. F. Hockey, Pathologist-in-charge, Laboratory of Plant Pathology, Kentville, Nova Scotia. The original purpose of the study was to determine for Kentville, Nova Scotia, the time during which the vegetative bud terminating the fruiting spur on the apple tree becomes differentiated into a flower bud.

Review of Literature

Date for the Earliest Initiation of Flowers

This is the first investigation of the subject for Nova Scotia, but a large number of investigators have reported on this point for other districts. Table I gives some of the dates that have been determined at other Stations for the earliest indication of flower bud formation.

It is evident that there is some variation in these dates, but it is reasonable to expect that there would be such a range, for there is a similar difference in the times for blossoming.

Criteria for Identifying the Earliest Stage of Flower Formation

Before the time of flower bud initiation could be determined, it was necessary to understand the morphological features by which the first stages could be recognized. Various descriptions of the first indication of flower formation

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TABLE I

DATES FOR THE EARLIEST FORMATION OF FLOWER BUDS

Investigator	Date	Place	Reference
Goff	June 30	Wisconsin	8, p. 298
Drinkard	June 20	Virginia	6, p. 167
Kraus	Latter part of June	Oregon	16, p. 18
Bradford	First ten days of July	Oregon	4, p. 5
Magness	About the last of June	Oregon	18, p. 4
Kirby	About the first of July	Iowa	14, p. 265
Tufts and Morrow	June 11	California	22, pp. 7 and 9
Ranker	June 19	Utah	20, p. 411
Rasmussen	July 19	New Hampshire	21, p. 255
Gibbs and Swarbrick	June 25	Bristol, England	7, pp. 63 and 65

are as follows:—*irregular contour of, or corrugations on, the crown* (Goff; 8, p. 294: Drinkard; 6, p. 167); *thickening of axis and rapid elevation of crown* (Kraus; 15, p. 5: Bradford; 4, p. 5: Magness; 17, p. 53: Rasmussen; 21, p. 258); *a flat crown* (Gibbs and Swarbrick; 7, p. 63); *a broad crown* (Kirby; 14, p. 274: Tufts and Morrow; 22, p. 6: Aaron; 1, p. 259). It is impossible to decide which author should be followed, for apparently they are not all describing the same structure. Also, from a study of the literature it is evident that most of the investigators take as the initial indication of flower formation, structures which are definitely flower or flower cluster primordia. By the time such a stage is reached the whole question of differentiation is finalized, and the tip is as certainly a flower bud as when the young flowers are completely formed some months later. It was felt that it might be possible to find some early stage in development, before the actual morphological appearance of the flowers, which would distinguish the potential flower-forming tip from the purely vegetative tip. Thus an intensive study of the development before and including the actual appearance of the flower was undertaken.

Length of Period During Which Flower Bud Initiation Occurs

In regard to the period during which flower buds may become differentiated, it is necessary to know not only the time when it starts but how long it lasts. On this there is disagreement among the authorities, some (Ranker, 20, p. 411; Gibbs and Swarbrick, 7, pp. 64 and 65) being of the opinion that flower bud initiation takes place at the first of the summer only and that the time during which it may occur is comparatively short and limited. Other investigators (Goff; 10, p. 311: Drinkard; 6, p. 204: Kraus; 16, p. 18: Bradford, 4, p. 5: Gourley; 12, p. 5: Magness; 17, p. 55: Kirby; 14, p. 265) consider that the initiation of flower bud formation continues all summer and even into the autumn. In addition, there are opinions given (Tufts and Morrow; 22, p. 10: Rasmussen; 21, p. 260) which are intermediate between these two extremes. So many investigators pronounced in favour of the protracted period, that the material collected during the latter part of the summer was examined very carefully to see if there was any evidence to support this view.

Structural Units of the Growing Tip

The leaves of the apple tree are produced in a spiral with a phyllotaxy fraction of two-fifths. The growth process which gives rise to such a structure is really quite complicated and a complete description must include detailed information on each of the morphological units of which the growing tip is composed. For convenience of reference these will now be listed and numbered under headings and sub-headings. *(1) The "crown" or pro-meristematic tissue at the tip.* To treat this structure adequately it must be studied from the following standpoints:—*(a), shape of the horizontal upper surface as seen in transverse section; (b), the contour of this surface as seen in longitudinal section; (c), the width of this surface and (d), the depth of the pro-meristematic tissue. (2) The leaf primordia and young leaves. (3) The pro-vascular tissue. (4) The cells developing into pith tissue.* Each of these structural units will be discussed under the headings as listed above.

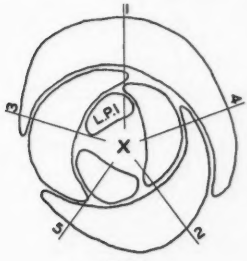


FIG. 1

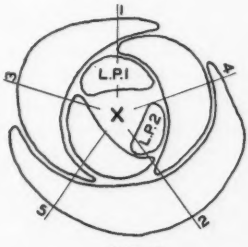


FIG. 2

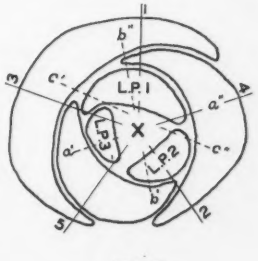


FIG. 3

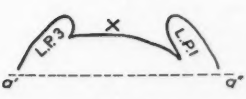


FIG. 4

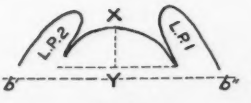


FIG. 5

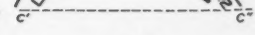


FIG. 6

FIGS. 1 – 6. FIGS. 1, 2, AND 3. *Diagrammatic horizontal projections of leaf primordia at the level of the crown-surface in a vegetative bud. These diagrams represent three successive stages in development. The leaf primordia are numbered according to their genetic sequence. The phyllotaxy of two-fifths is indicated by the radial lines 1 to 5. L.P., leaf primordium; X, centre of crown.* FIGS. 4, 5, AND 6. *Enlarged longitudinal sections through a'–a'', b'–b'' and c'–c'' (Fig. 3) respectively; L.P., leaf primordium; X, centre of crown; X–Y (Fig. 5) distance which the base of leaf primordium No. 3 is above the bases of leaf primordium No. 1 (Fig. 3).*

1a. Shape of the Crown as Seen in Transverse Section

The shape of the horizontal upper surface of the promeristem is roughly an equilateral triangle, but it is very difficult to describe this area, for it is constantly undergoing changes which are varied, complex and continuous. In Figs. 1 to 3 this growth process is illustrated by horizontal projections of the tip in three successive stages, but the best way to represent it is to make a model of the tip at any one stage and then rotate this model through 720 degrees, stopping at each of the five points 144 degrees apart (axes 1, 2, 3, 4,

and 5, Figs. 1 to 3). The model when stopped at each of these five points will illustrate the progressive development of the tip. However, in spite of the many changes that occur over the surface of this crown, there is one feature which is constant and characteristic of purely vegetative growth, that is, the roughly triangular outline of the horizontal upper surface.

1b. Contour of the Crown as Seen in Longitudinal Section

For any one growing tip the contour of the upper surface depends upon the plane in which the longitudinal section is cut. Figs. 3 to 6 illustrate this point. It will be seen that longitudinal sections through a′–a″ and b′–b″, Fig. 3, would both be median, but a′–a″ would have a contour as shown in Fig. 4, whereas the tip through b′–b″ would appear as illustrated in Fig. 5. An extreme and incorrect conception of the tip would be obtained by a section through c′–c″, Fig. 3, when the tip would appear to have a broad flat crown as indicated in Fig. 6. Thus, depending on which median plane is used and whether the plane of the cut is median or slightly lateral, the one growing tip might give a great variety of outlines for the external surface of the crown as seen in longitudinal section.

The sections most useful for indicating the characteristic contour were the median ones. In this work these significant sections were determined by counting the number of sections that included the pro-meristematic tissue of the crown. Then those chosen as most likely to represent the true centre of the bud were the central numbers of the series. If there were 17 sections showing pro-meristematic tissue, Nos. 8, 9, and 10 were considered as significant or central for that growing tip. It is evident that a continuous and complete series is absolutely necessary for such a determination. Failure to follow this procedure led to grave errors. Hence, unless otherwise stated, all drawings of growing tips in the present paper are from sections selected as described above.

Respecting the information that can be gained from a study of these contours, the median longitudinal section cut through the plane b′–b″, Fig. 3, and giving the usual convex outline illustrated in Fig. 5, is perhaps the most significant. For the central point, "X" (Figs. 1 to 5) is always on a level with the base of the youngest leaf primordium (see L.P. 3; Fig. 4); thus the distance X–Y, Fig. 5, represents fairly well the amount of new growth at the tip since the formation of leaf primordium No. 1. Hence a decidedly convex crown in such a section suggests rapid growth, and conversely a flatter crown in the same type of longitudinal section suggests a slowing down of growth in length. This is by no means an infallible guide, but it is helpful. Due to the significance of this type of section, the terms "convex", "flat", etc., when used in the present article, apply to the contour of the crown as seen in a median longitudinal section through a plane similar to b′–b″, Fig. 3.

1c. Width of the Crown

There are frequent references in the literature to the "width" of the pro-meristematic area, but what is the width of a triangle the outline of which

is not continuous? In some articles this width is indicated in microns by a figure which was arrived at by making one measurement for each tip. Any attempt to indicate the width of the crown by one such measurement may be misleading. What is required is not the size of the crown in one growing tip, but a means by which the size of the crown in one tip may be compared with the size of the crown in some other tip. It was found that the most accurate indication of comparative sizes was obtained from the average of three measurements. This average is called the "Index of Crown Width". To obtain the necessary data to compute this index, complete series of longitudinal sections were used and three measurements taken as follows: (i) by multiplying by ten the number of sections showing pro-meristematic tissue (the sections were cut 10μ thick); (ii) by using the median section of this series and measuring in microns the distance from the youngest primordium base of one side to the youngest primordium base of the opposite side; (iii) by using the section through that tip which showed the greatest pro-meristematic width, and making for it a measurement similar to that made in (ii). The average of these three figures was then considered as the "Index of Crown Width" for that bud. The index given for each week is the average of the indices of all the buds collected during that week. The number of buds examined for each week ranged from two to eight. This index must not be considered as an accurate representation of size, but merely a comparative indication.

Instead of using the crown width index as an indication of crown size, an attempt was made to use the area of the upper surface of the pro-meristematic region and to compute this area from transverse sections, but it was impossible to decide when the youngest primordium should be included in the area and when it should not. There is no sudden change in the natural development from unquestioned terminal pro-meristematic tissue to definite and distinctly marginal primordia. Any arbitrary division led to misconceptions, so this plan was abandoned.

1d. The Depth of the Pro-meristematic Tissue

The vertical depth of the pro-meristematic tissue at the tip varies greatly from season to season. The measurements for this were made in the median longitudinal section and represent the distance in microns from the outer surface of the centre to the first cells showing definite vacuolation, preparatory to enlarging into pith cells. The inner limits for this measurement are obviously indefinite, but the figures will be an aid in comprehending the changes in structure at the tip.

2. Leaf Primordia and Young Leaves

In studying the leaf primordia and young leaves the most important point to note is the position of their bases in respect to the level of the surface of the crown, that is, whether many of the bases are on the same level with the pro-meristematic tissue or most of them lower down. Of those that are lower down, the vertical distance between the bases affords an excellent indication of the rate and extent of growth.

3 and 4. Pro-vascular Tissue and Pith

With both the pro-vascular tissue and the pith, the significant feature is the outline of the distal portions as seen in median longitudinal section. The terms used for describing this are, "acute conical", "broad hemispherical", and "narrow cylindrical", etc. These are self-explanatory.

Distinction Between Vegetative Buds and Flower-forming Buds

To interpret the early stages correctly, it was of paramount importance to be able to differentiate potential flower buds from those that were potentially vegetative only. During the first part of this investigation, all collections were made from the McIntosh Red. The bearing habits of this variety are such that one cannot always foretell with accuracy which buds will remain vegetative and which will develop into flower buds. The only way to secure buds that could be classified in this way, with a minimum chance of error, was to have trees on which, during one season, the main bud of the fruit spur was of one type only. Ordinary "off" and "on" trees (so called) would not be suitable, for most varieties which are casually termed "biennial bearers" really have a heavy crop followed by a light crop. A search of the records of the orchard showed that certain Wageners were very heavy bearers every second year and really completely "off" during the alternate years. The two most suitable Wageners were selected, and by taking buds from these trees only, the possibility of error at the time of collection was reduced to a minimum.

When these collections were made, buds were not taken indiscriminately, but the data provided by such workers as Crow (5), Maney and Plagge (19), and Hooker (13) were taken into consideration and all collections were made from spurs of medium length. Also, when collecting from the "off" tree, buds were taken from spurs that during the previous year had produced a medium amount of growth and had actually borne fruit. Similarly, when collecting axillary buds from the "on" tree, long whip-like or stunted growths were avoided, and material was taken only from spurs actually bearing blossoms or fruit. In this way it was hoped to limit the study as far as possible to growth that would have produced fruit in the normal biennial cycle.

Methods and Technique

During this investigation various methods were employed. Firstly, both longitudinal and transverse sections in series were obtained. Using a complete longitudinal series through a bud, camera lucida drawings of each section were made on sheets of wax. These drawings were cut out and built into models. As the wax had been poured to a predetermined thickness, correct proportions were obtained for the models. These models were of assistance, but the greatest aid was to dissect, stain and examine the young tip in the way described by Gore (11). To get a satisfactory dissection it was necessary to use the special needles described by Henderson (Bell and Facey, 2. pp. 130–131) and to soak the bud for 24 hr. in 70% alcohol before dissecting. Fresh buds were too soft to dissect and pure alcohol made them too brittle.

Just before completing the final stages of the dissection, the bud had to be stained in the tincture of iodine, otherwise the smallest leaf primordia could not be seen clearly and might be injured by the needles. The completely dissected tip, stained in tincture of iodine, placed in a ray of very strong light and examined under a magnification of at least 150 diameters, revealed details of structure which could be seen clearly by no other method. For microtome work, material was killed, imbedded, and sectioned as described by Bell and Facey (2). The sections were cut 10 μ thick. The stain that proved most satisfactory was a combination of safranin and fast green.

Frequency of Collections

With both the McIntosh and Wagener, the collections were made at least twice each week from April to October inclusive, twice a month during November and March, and once a month during December, January, and February. For each collection an average of 12 buds was taken from scattered parts of the tree. The collections of McIntosh were started in April 1934, but discontinued late in the summer of 1936. The collections of Wagener were continuous from April 1936 to September 1938.

The Development of the Growing Tip

Explanation of Terms Used

A short explanation is necessary regarding the terms used in the following description of development. Firstly, the mature apple is the climax and end of a growth that was initiated over three years prior to the date on which the apple is picked. It is obvious that developmental cycles overlap and at any time more than one stage may be found on one tree. To avoid confusion, the development of one tip will be followed through from start to finish. It would be misleading to use dates. To avoid their use, the season during which the tip first becomes differentiated as a separate organ will be referred to as year "I", the next year as "II", and so on. To correlate the symbols I, II, etc., with the terms "off" and "on", year I is an "off" year; II, "on"; III, "off"; and IV, "on". To make the description clearer, the development will be dealt with in what appeared to be natural "phases", but this is not meant to indicate that there is any sharp transition between one phase and the next, for of course development is continuous.

PHASE 1. Initiation of a New Growing Tip

From summer of year I ("off") to winter of years I ("off") and II ("on"). Figs. 10 and 18a.

Year I, for the particular spur under discussion, is an "off" season. During the summer of this year I, there is matured at the terminus of the spur a mixed bud containing the primordia of about five flowers surrounded by five to seven leaves. As usual, in the axil of each young leaf, is a small group of embryonic cells (potential growing tips). Towards the end of the summer, the embryonic tissue in the axil of at least one of the leaves (which is usually

TABLE II

INDEX OF CROWN WIDTH AND DEPTH OF PRO-MERISTEMATIC TISSUE

Year II, "On"			Year II, "On"—*Concluded*		
Collection date	Index of crown width	Depth of pro-meristematic tissue, μ	Collection date	Index of crown width	Depth of pro-meristematic tissue, μ
April 29	88	113	Nov. 4	76	124
May 2	95	113	19	94	128
4	111	94	Dec. 10	72	134
5	70	101	Year III, "Off"		
6	74	103	Collection date	Index of crown width	Depth of pro-meristematic tissue, μ
7	121	94	Jan. 20	111	129
8	95	106	Feb. 5	83	137
9	120	99	Mar. 5	129	149
14	124	97	19	104	142
18	104	103	April 2	87	134
21	149	105	12	102	142
25	123	110	21	97	135
28	135	99	28	92	124
June 1	124	113	29	80	103
4	153	101	May 2	105	94
6	144	97	4	121	92
8	143	90	5	108	112
11	108	85	6	100	97
13	142	89	8	120	106
15	163	76	11	129	106
18	179	86	14	121	88
20	149	97	18	121	103
22	158	83	19	87	80
24	124	83	21	100	80
25	106	71	25	115	89
27	164	97	26	115	80
29	138	93	28	170	97
July 1	136	87	30	127	84
2	158	83	June 1	199	97
4	159	112	4	203	79
5	181	71	6	145	84
6	141	84	8	209	97
7	143	101	11	142	80
8	110	71	13	167	93
11	144	84	15	134	84
12	184	80	18	143	86
13	143	93	20	207	89
15	132	100	22	148	89
18	114	97	25	200	89
19	152	103	27	165	85
22	136	89	29	155	71
26	129	89	July 2	210	97
27	145	89	4	158	89
29	136	89	6	164	97
Aug. 1	134	94	7	151	93
2	161	93	11	152	101
5	131	97	13	152	97
8	147	88	15	134	97
15	120	80	18	145	95
22	112	92	20	153	88
29	118	94	22	164	103
Sept. 3	121	93	25	197	130
15	139	103	27	182	128
20	101	112	29	171	101
Oct. 4	93	127			
20	102	119			

neither the highest nor the lowest) has developed into a definite growing tip with at least two leaf primordia of its own (Figs. 10 and 18a). It is the development of this tip which will be traced from its inception during the the summer of year I until its individuality as a growing tip ceases, with the formation of flowers. By the end of August, year I, this young growing tip is quite easily identified with the aid of a magnifying glass. In composition its cells are practically all embryonic, those of the young leaves being differentiated only slightly. The crown is narrow and convex. In this condition it overwinters during the winter of years I and II.

PHASE 2. Development of a Broad Crown in the Vegetative Bud

From the early spring of year II ("on") to late July of year II ("on"). Figs. 11, 19, and 20a.

When activity of this axillary growth starts in the spring of year II its crown is at first narrow and decidedly convex. The pro-meristematic tissue is medium in depth and neither the pro-vascular strands nor the pith are distinctly differentiated. Before the expansion of the blossoms, this new growth is enclosed within the flower bud and is difficult to locate without the aid of a magnifying glass. During the latter part of May it grows in length, and by the time of full bloom it may be seen as a very short lateral branch, emerging from the axil of one of the leaves (Fig. 19). The leaves formed by this new and axillary growing tip become matured just after those which surround the flower cluster. This new growth is then established as one of the gross morphological features of the spur.

By this time (the middle of June) a number of changes have taken place in the growing tip of this new lateral branch. The crown has broadened, though it is still convex. The leaf primordia are below the surface of the crown. The pro-meristematic tissue is not as deep as it was, but is still of medium depth. The pro-vascular strands and pith are completely differentiated and are narrowly hemispherical in outline. Through the latter part of June and through July the development continues, and reaches its climax late in July (Figs. 11 and 20a). By that time the average crown width index is about 140. Some crowns may be very broad, attaining a width index of 181. It is still convex in outline, though often flatter than it was during June and early July. The pro-meristematic tissue has become very shallow, often being reduced to a depth of 80 microns. The bases of the young leaves and scales approach the same level as the pro-meristematic tissue. Both the pro-vascular strands and the pith are broadly hemispherical in outline.

PHASE 3. Development of the Acutely Conical Internal Structure of the Winter Bud

From late July of year II ("on") to the winter of years II ("on") and III ("off"). Figs. 12 and 20b.

After the last of July, the tip continues to grow, but the growth between this time and winter produces a structure which is different from that found at the tip during any other phase of its development. To understand this

structure it must be remembered that by the first of August, when this new phase starts, the scales of the winter bud are almost mature and quite rigid. Growth then is limited to the internal or central portions of the bud. During this phase of development the surface of the crown rises well above the bases of the outer and larger bud scales, the width of the crown gradually narrows, its outline becomes less convex and the depth of the pro-meristematic tissue increases. The outlines of the pro-vascular strands and pith change from hemispherical to conical. An early stage may be seen by the middle of September. At this time the internal and active portions of the bud taken as a whole form a structure like a pyramid or broad-based cone. The climax of this phase is reached by November (Figs. 12 and 20b), and then the structure of the tip remains unchanged in its characteristic features throughout the winter. During this time (the winter of years II and III) the crown is so narrow that it may have a width index of only 73. A young primordium is always present. Its base is level with the upper surface of the crown. This upper surface of the crown is flat and at its periphery it slopes abruptly down to the base of the second youngest primordium. The base of this second youngest primordium is decidedly below the base of the youngest primordium and the surface of the crown. The pro-meristematic tissue may be 145 μ in depth, which is almost double that of any other stage. The pro-vascular strands and pith are acutely conical. All the internal portions of the bud now form a structure like a long, narrow or acute cone, with the crown and youngest primordium at the apex and the other primordia and young leaves arranged in a spiral down the steeply sloping sides. It is difficult to interpret the significance of all these features. The flat crown suggests inhibition of growth, but the increased vertical distance between the bases of the primordia, and the great increase in depth of the pro-meristematic tissue, indicate that some growth is taking place. The great depth of the pro-meristematic tissue suggests also that either differentiation is not taking place or is taking place very slowly. Certain median longitudinal sections of the tip at this stage, if taken by themselves, may lead to a false conception of tip structure. Should the section not include the youngest primordium, the crown will appear as a narrow, flat-topped, raised plateau. The whole appearance of the internal structure during this period gives the impression that the tip is growing within a restricted space and that the only outlet for its increasing length is to push up a narrow conical growth between the more or less rigid and inactive bud scales. Sections of buds from the December, January, February, and March collections suggest that a very slow growth and slight increase in size continues even during these months, but as yet this cannot be stated with certainty. In its characteristic features the form which the tip assumed by November, year II, remains almost unchanged until about the middle of March, year III.

PHASE 4. Rapid Elongation During the Early Spring of Year III

From early spring of year III ("off") to foliation during the last week of May, year III ("off"). Fig. 13.

Growth activity starts within the bud very early in the spring (year III,

"off"). Actual cell division is observed early in April. This is before there is any great change in the shape or size of the bud. During April and May the growth of the axis inside the bud is very rapid and produces a structure which is unlike that of any other stage (Fig. 13). The unique feature of this phase is due to a rapid growth in length which is not accompanied by a corresponding increase in diameter, hence the axis is pushed out into a long narrow cylinder. The crown becomes slightly broader and regains its convex outline. It proliferates young leaves and scales so rapidly that there is no great vertical distance between the bases of the two or three youngest primordia, but, due to the very rapid elongation of the axis just below the tip, the bases of the slightly older primordia and young leaves become very widely separated vertically. The usually fairly sharp boundary between embryonic cells and young pith cells is entirely lost and there is a long region in which the structure of the tissue is halfway between pro-meristem and pith. The strictly pro-meristematic tissue is not as deep as it was during the winter. Both the pro-vascular tissue and the pith are narrowly cylindrical. During this phase the long distance between the bases of the young leaves, their conspicuous and curved leaf traces, the parallel sides of the long narrow pro-vascular cylinder, and the long transitional region between pro-meristem and pith give to the median longitudinal section a decidedly unique and characteristic appearance.

PHASE 5. Broadening and Flattening of the Crown Previous to Flower Formation

From foliation during the last week of May, year III ("off"), to the latter part of July and first week of August, year III ("off"). Figs. 14 and 22a.

About the end of May the new and attenuated axis starts to broaden throughout its whole length. This is just about the time of full bloom for the "on" spur and the maturing and expanding of the leaves for all spurs. Early in June this broadening of the axis continues and is accompanied by decided and very rapid changes at the tip. Of these changes, the most conspicuous is in the width of the crown. It becomes very broad, attaining an average width index of 165, but some may reach a width index of 250. The contour of the crown is much less convex. Sometimes it is almost flat. The pro-meristematic tissue becomes very shallow, often having a depth of not more than 70μ. Most of the bases of the leaf primordia are level with the pro-meristematic region, giving the whole tip a "broad-shouldered" appearance in longitudinal section. The pro-vascular strands and pith are both broadly hemispherical in outline. The climax of this phase of development is reached during the first weeks of July, year III (Figs. 14 and 22a). At this stage it resembles the tip of the last of July, year II, except that it may be a little flatter, but it is decidedly broader and flatter than the tip of the same date (first week of July) year II. Wording this another way: by the first of July, the growing tip of the "off" spur is broader and flatter than the growing tip of the axillary branch of the "on" spur, but it is similar to the shape that the

tip of the axillary branch of the "on" spur will assume by the last of July. The tip that has just been described for early July, year III ("off"), with its broad flat crown, its shallow pro-meristematic tissue, its leaf primordium bases level with the pro-meristem and both pro-vascular strands and pith broadly hemispherical, is the structure that immediately precedes the initiation of flower primordia. It is found on the "off" spur during the first three weeks of July, year III.

PHASE 6. Initiation of Flower Primordia

During the last two weeks of July or the first week of August, year III ("off").

Sometime during the last two weeks of July or the first week of August the formation of leaf primordia ceases and the five sepal primordia of a terminal flower are formed instead. In the proliferation of leaf primordia, during purely vegetative growth, the last-formed primordium always becomes elevated above those formed previously. During the development of the five sepal primordia, this elevation does not occur, consequently they all appear at the same level; that is, in the same horizontal plane. The result is, the shape of the crown as seen in transverse section changes from triangular to circular. This change in the shape of the upper surface of the crown and the appearance of the five sepal primordia are the first morphological indications of the initiation of flower formation.

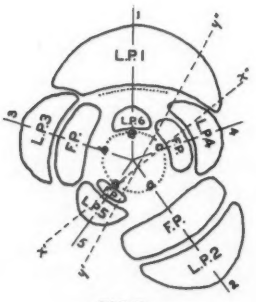


FIG.7

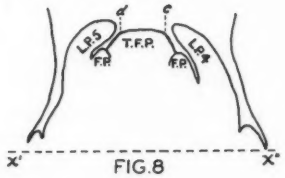


FIG.8

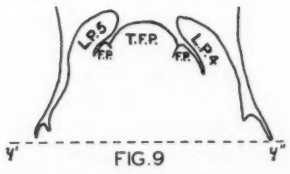
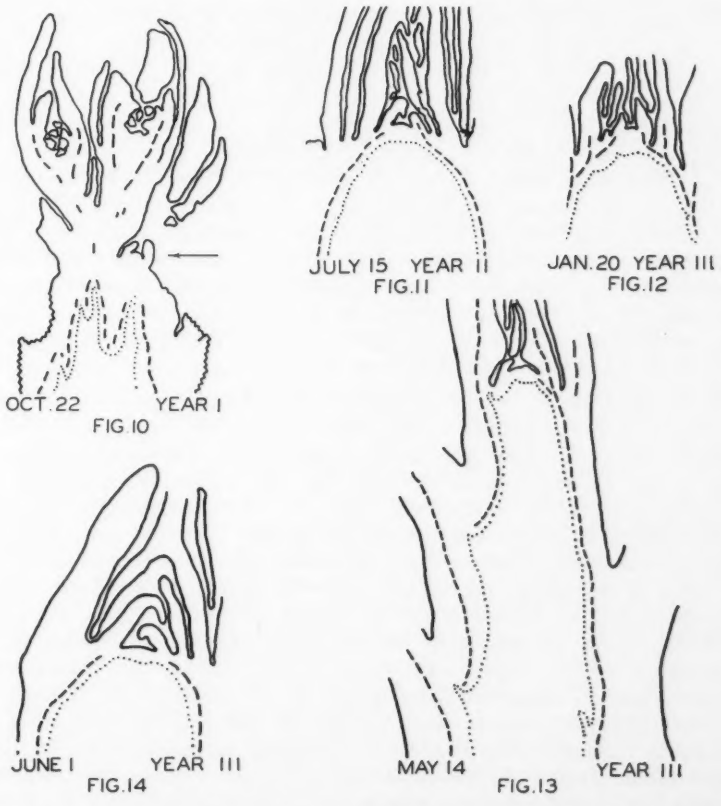


FIG.9

FIGS. 7 – 9. FIG. 7. *Diagrammatic horizontal projection of a flower-forming growing tip; L.P., leaf primordium; F.P., flower primordium; a, b, c, d, and e, points at which sepal primordia are appearing.* FIGS. 8 AND 9. *Enlarged longitudinal sections through x'–x'' and y'–y'' (Fig. 7) respectively; L.P., leaf primordium; F.P., flower primordium; T.F.P., terminal flower primordium; c and d, sepal primordia.*

The change from a triangular to a circular crown occurs very rapidly. Considering leaf primordium No. 6, Fig. 7, as the last leaf primordium formed, the five sepal primordia will appear at points *a*, *b*, *c*, *d*, and *e* in a circle at the periphery of the crown. The one at *a* appears first, then, in rapid succession,

b and *c*. About the same time as *a* and *b* appear, but before the appearance of *c*, the tissue towards the periphery of the crown rises slightly. This rise occurs between *a* and *b* and also beyond *b* towards the place where *c* will appear. This raised margin, as seen in cross section, is not straight but is curved. Thus, at this stage the margin of the crown from *a* to, and beyond, *b* forms a semicircle. From *c* to *a* the margin is still practically a straight line. Primordium *c* then appears and the elevated circular margin is continued to *a*. Almost immediately, primordia *d* and *e* appear. At this initial stage, primordium *a* is the largest and *e* the smallest. Their gradation in size is represented by the alphabetical order. The crown is now circular in outline (dotted circle, Fig. 7). The five sepal primordia, and the slightly raised mass of pro-



FIGS. 10 – 14. *Camera lucida drawings of the median longitudinal section through the growing tip. Date of collection is indicated on each figure. Pro-vascular strands indicated by dashes; outline of pith indicated by dots.* FIG. 10. *Arrow points to primordium of new axillary branch. Magnification 23.*

meristematic tissue from which these sepal primordia originate, form the primordium of the terminal flower.

In a longitudinal section of the tip at this stage the crown is seen to be either very slightly convex or quite flat. As the whole crown is raised without very much broadening of its base, the sides slope very steeply. In a longitudinal section through the sepal primordia (along line x′–x″, Fig. 7) the upper surface of the crown curves abruptly through an angle of about 45° to the steeply sloping surface of the side (Fig. 8). In sections between the sepal primordia (along line y′–y″, Fig. 7) the upper surface curves more gradually to the side (Fig. 9). These sepal primordia are not conspicuous in longitudinal section because, in the very early stages, they are not raised above the centre of the crown but merely form undulations at its periphery.

To observe the various steps in the appearance of the sepal primordia it is necessary to examine stained dissected buds under a compound microscope, and while the dissected bud is being examined in the ray of very strong light it must be rotated through 360°, otherwise shadows may give to the crown an appearance which is quite misleading. A good dissecting microscope is not suitable for these observations because its depth of focus is too great. Very slight elevations are not apparent, and the first appearance of the sepal primordia will not be observed. With the compound microscope and a sufficiently high magnification, the tips of the sepal primordia may be brought into focus, with the tissue between them out of focus. This tissue between the embryonic sepals will come into focus if the lenses are lowered very slightly. By this method minute elevations on the surface of the crown may be detected and the five sepal primordia at the periphery of the crown may be identified at a very early stage.

While the central and terminal flower is being differentiated, the primordia of the lateral flowers appear. These lateral flowers are all axillary and were observed about the same time as the appearance of primordium *c*, that is, while the crown was becoming circular. At first these axillary primordia are linear, tangentially elongated elevations of pro-meristematic tissue and appear in the axils of leaf primordia Nos. 2, 3, 4, and 5. The very early stages of their development provide the corrugations seen in longitudinal section by Goff (8). Longitudinal sections through x′–x″ or y′–y″, Fig. 7, would show a condition and are illustrated in Figs. 8 and 9. The flower primordia in the axils of the leaf primordia are pressed tightly against the terminal flower primordium and as they are all pro-meristematic tissue the terminal and lateral primordia appear, in longitudinal section, to have had a common origin, namely, the crown. But in the dissected buds the lateral flower primordia are seen to be separate and axillary structures (Fig. 7). The flower primordium in the axil of leaf primordium No. 2 may be recognized first, and in the early stages it is the largest of these lateral flower primordia. The others grade in size to that in the axil of leaf primordium No. 5, which is sometimes difficult to recognize in the very early stages. Narrow elongated zones of pro-meristematic tissue were recognized in the axils of leaf primordia

Nos. 6 and 1, but in the specimens examined these had not developed into flower primordia.

About the same time as the appearance of the sepal primordia *d* and *e* (Fig. 7), and when the axillary flower primordia are first apparent, all the flower-forming tissue becomes elevated very rapidly (Figs. 8, 9). The primordium of the terminal flower is always above the others. Its upper surface becomes slightly concave or saucer-shaped, but it is always circular as seen in cross section. The pro-vascular strands and pith follow into the elongating tip and become acutely conical in outline as seen in longitudinal section. This is probably the stage described by Bradford (4) and Magness (17) as the first indication of flower formation, and as the distal portion of the pro-meristemic tissue is not yet differentiated, this may also be the stage described by Kraus (15) as a "thickening of the axis", for the undifferentiated embryonic tissue could be considered as "thickened" whether it be measured horizontally or vertically. Finally, after further elevation, the terminal flower primordium becomes distinctly raised and develops a decidedly concave upper surface. The flower primordia in the axils of leaf primordia Nos. 2, 3, 4, and 5 develop into laterally compressed flower primordia. From the standpoint of gross morphology, the whole terminal structure is now established as an embryonic flower cluster.

From the description given above it is seen that the development of the terminal flower primordium is initiated slightly in advance of the others, but later proceeds concurrently with the development of the lateral flower primordia. In both, all stages follow each other rapidly, and only by dissecting a large number of buds, during the critical period, may all be seen.

It is evident that on the appearance of sepal primordium *e* (Fig. 7), the crown has ceased to exist as an apical organ of vegetative growth, and all its tissue has become differentiated into the terminal flower primordium. There is no question of there being any axillary pro-meristematic tissue in the structure of this terminal flower primordium, for after it is completely formed the axillary embryonic tissue may still be recognized in the axil of leaf primordium No. 6. Also, the terminal flower primordium is differentiated before the lateral flower primordia. This is in agreement with the findings reported by Black (3, p. 527) but is not in agreement with the statement made by Bradford (4, p. 5) that "The apical protuberance is differentiated last . . . .". The findings reported in the present investigation suggest that the inflorescence of the apple is "determinate", but that the order of development for the other flowers of the cluster, namely, those in the axils of leaf primordia Nos. 2, 3, 4, and 5, Fig. 7, is acropetal and not basipetal.

The time at which this development occurred varied with the variety. During one season with the McIntosh, these stages appeared by the third week of July; in a different season, with the Gravenstein, they occurred during the fourth week of July, and with the Wagener during the first week of August. However, for any one variety in any one season, differentiation was started and completed within a very short period, not more than two weeks. After

this period, during which the embryonic flower cluster stage was reached, no new initial stages of flower formation were observed.

Later Stages of Development

Later development includes completion of flower formation (Fig. 22b) expansion at blooming (Fig. 23), the setting, maturing, and ripening of the fruit (Fig. 24). All these stages have been excellently described by many investigators, as Drinkard (6), Kraus (15), Bradford (4), etc. Thus there is no need to repeat the description in this article.

The complete story for one growing tip is represented diagrammatically by Figs. 18 to 24. These should be of assistance in associating the steps in tip development, as seen under the microscope, with the familiar external appearance of the spur at each of the various stages. Crown width indices and measurements of depth of pro-meristematic tissue are given in Table II. In Figs. 15 and 16 the weekly averages from the two tables are plotted. A comparison of the two graphs suggests that on the average a broad crown is associated with the shallow pro-meristematic tissue and a narrow crown with deep pro-meristematic tissue.

Discussion

Some statements have already been made in explanation of the stages that former investigators have reported as the first indication of flower formation. These investigators do not report the shape of the crown as seen in transverse section changing from triangular to circular, nor do they correlate this with the first appearance of the sepal primordia, but Goff (8) described the appearance of this stage as seen in longitudinal section. The broad crown taken as the first indication of flower formation by Kirby (14), Tufts and Morrow (22), and Aaron (1), and the flat crown used by Gibbs and Swarbrick (7), are not sufficient, for about the last of July the purely vegetative tip will also have a broad and fairly flat crown. The thick and non-serial sections used by Rasmussen (21) would not be sufficient for an accurate determination of the earliest stages.

It can be stated as a general principle that many methods and many types of section are needed before one can make an accurate identification of the first morphological indication of flower formation.

As already stated, the period during which this initiation of flowers occurs is not extensive and does not last until late in the summer. However, there must be some real foundation for the many statements in the literature that the period is of long duration and may extend into the autumn. There are two probable explanations. First, in cases of abnormal development (and these are very common in plants) a structure may appear completely out of season. Some of the very late instances of flower initiation were probably of this nature. The second explanation is that occasionally some vegetative buds on the "on" tree have, during August, very broad crowns. When these become elevated, preparatory to the formation of the conical winter structure,

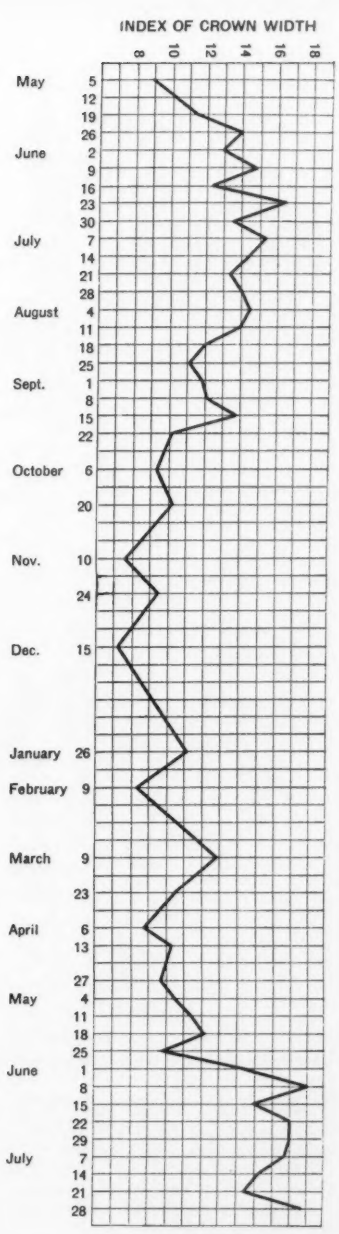


FIG. 15

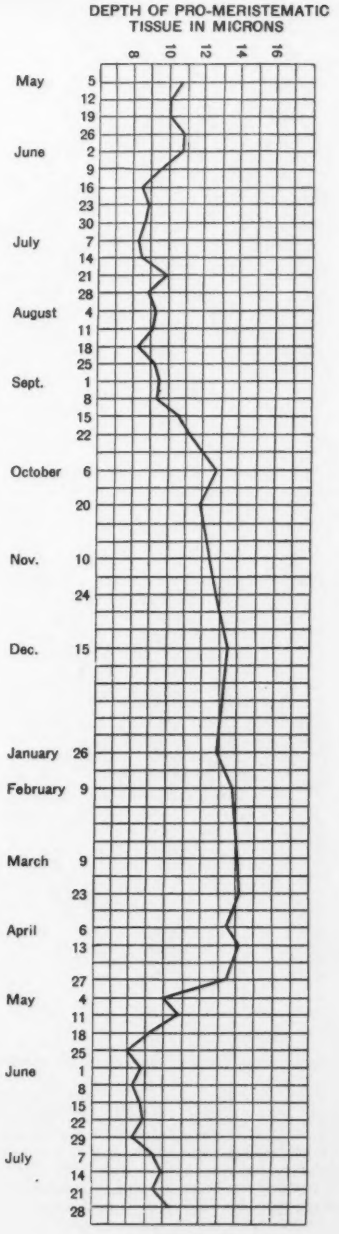
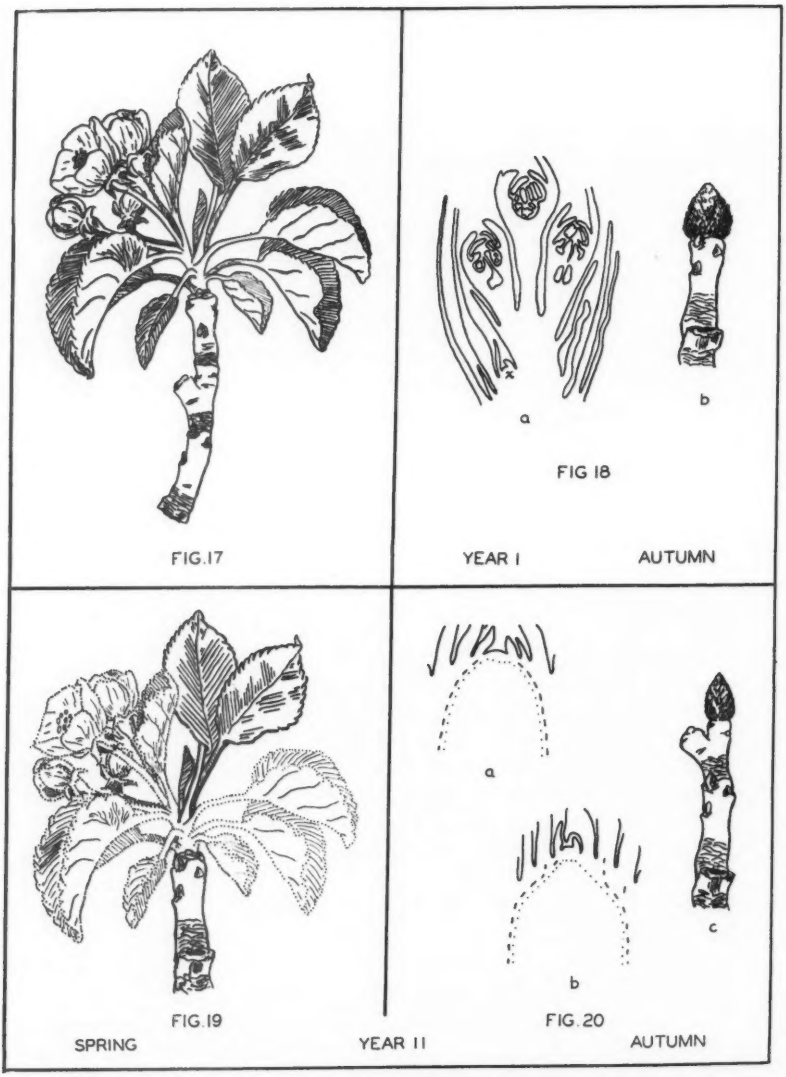
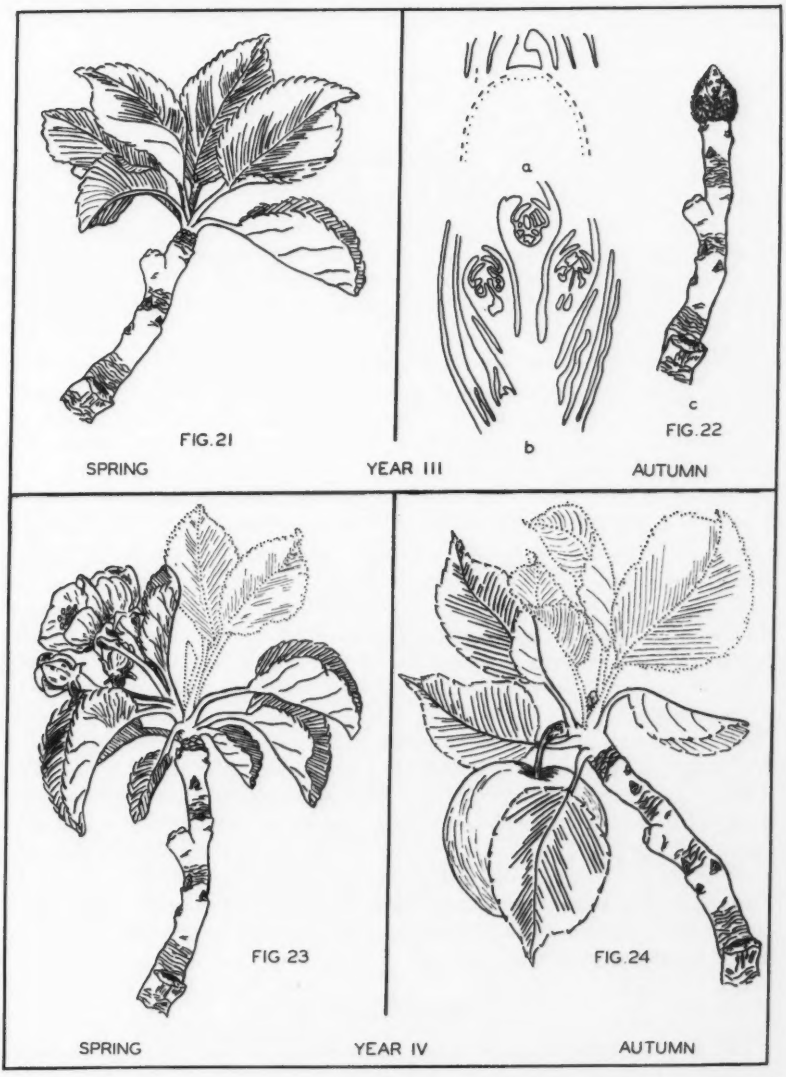


FIG. 16

FIGS. 15 AND 16. FIG. 15. *Crown width index, plotted on a basis of the weekly average.* FIG. 16. *Depth of pro-meristematic tissue, measured in microns and plotted on a basis of the weekly average.*



FIGS. 17 – 20. FIG. 17. *Typical biennially bearing fruit spur, illustrating flower cluster, young lateral branch and annual growth in length of spur.* (FIGS. 18 – 24. *The development of a single spur from late autumn year I, to harvest time, autumn year IV.*) FIG. 18. *Winter bud (mixed) autumn year I "off".* a, *longitudinal section;* x, *new axillary growth;* b, *habit sketch of bud and spur.* FIG. 19. *Spur at blossom time, spring year II "on". Flower cluster and its leaves stippled, new lateral shoot lined.* FIG. 20. *Development of new lateral shoot, year II "on".* a, *longitudinal section of mid-summer tip;* b *and* c, *late autumn;* b, *longitudinal section of tip;* c, *habit sketch of winter bud (leaf) and spur.*



FIGS. 21 – 24. FIG. 21. *Spur at time of foliation, spring, year III "off".* FIG. 22. *Flower bud and spur, year III "off".* a, *tip at end of June or first of July, i.e., just prior to flower formation;* b *and* c, *autumn;* b, *longitudinal section of bud;* c, *habit of sketch of bud and spur.* FIG. 23. *Spur at blossom time, spring, year IV "on". Flower cluster and its leaves lined, new lateral shoot stippled.* FIG. 24. *Spur at harvest time, autumn, year IV "on". Fruit and the leaves that expanded with flower cluster, lined; new lateral shoot and its leaves, stippled.*

their appearance in longitudinal section is similar to that of the potential flower bud at the commencement of flower formation when elevation is first apparent. It is possible that the late summer stage of these vegetative buds may have been mistaken for the early stage of flower initiation.

Conclusion

In conclusion, reference should be made to the original purpose of this investigation, namely, the first morphological indication of flower differentiation. This might have reference to merely the first appearance of a structure which will develop into a part of the flower, or to a change in structure which, though not a stage in flower formation, could be taken as an indication that physiological differentiation of a fruiting tip is in progress. In the first and purely morphological aspect the purpose of the investigation has been fulfilled. The first morphological indications of flower differentiation were found to be the change in shape of the upper surface of the crown, as seen in transverse section, from triangular to circular and the appearance of the five sepal primordia, and this takes place during the last two weeks of July or the first week of August. This is of interest from the standpoint of morphology, but, to one wishing to influence flower differentiation, it is of little interest, for physiological differentiation must precede morphological differentiation. The crown becoming circular indicates that the question of flower differentiation is settled, and probably cannot be influenced very much one way or the other. Thus, for those who wish to influence flower initiation, it is much more important to discover a morphological change that would indicate that physiological differentiation is in progress. The detailed account of tip development given above may be some help in the discovery of this. The authors wish to make a suggestion. The major morphological changes that immediately precede the initiation of flower primordia are those described in Phase 5, and commence in the "off" bud during the first two weeks of June or just after foliation. In the vegetative bud a similar change does not occur until over a month later. It is suggested that this early broadening and flattening of the crown in the "off" bud during the early part of June may be a morphological indication that physiological differentiation is taking place. Of course, this is only a suggestion, for, before it can be proposed even as a theory, experimentation is necessary.

Acknowledgments

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OBSERVATIONS ON THE BIONOMICS OF OVA AND MIRACIDIA OF *FASCIOLA HEPATICA* LINN., IN EASTERN CANADA[1]

BY HENRY J. GRIFFITHS[2]

Abstract

The effect of temperature, light, darkness, and chemicals on the hatching of the eggs of *Fasciola hepatica* is discussed. Some malformations of the eggs of this parasite together with observations on the miracidia are recorded. A description of methods employed for attempted infestation of suspected snails is given. In no instance, however, were any specimens of the 11 species of snails exposed, proven to act as intermediate host for *F. hepatica*.

Introduction

During an investigation of the incidence of *Fasciola hepatica* Linn. in the lower St. Lawrence valley, a number of observations was made on the bionomics of the micacidia and ova of this parasite. The results, together with the findings of other investigators, are presented in the following paper.

In order to carry on experimental tests on the ova of *F. hepatica*, it is highly desirable that a readily accessible supply of material be available. It is perferable to have pure cultures of eggs direct from the gall bladder of the infected host. The contents of the gall bladder should be emptied into a glass container and the eggs allowed to sediment in water. After 15 min. the supernatant fluid may be poured off and the jar refilled with water. This procedure should be carried on until all traces of bile are removed.

The eggs may then be left for further development or held in a refrigerator at 2 to 4° C. until development is required. Ova may be obtained directly from the faeces of a heavily infected individual by screening, sedimenting and washing to remove all soluble matter; this method, however, is very tedious unless the host animal is very heavily infected.

Effects of Temperature, Light, Darkness, and Chemicals on the Hatching of Ova

In the course of his classical research on the life cycle of *F. hepatica*, Thomas (9) found that temperature played an important role in controlling the rate of development of the eggs. The optimum temperature was about

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council.

[2] *Assistant.*

22 to 26° C., at which development took about 14 days; at 16 to 18° C., development took two or three months, while none took place at less than 10° C. The influence of temperature on the rate of development of fluke eggs is of practical importance, since it probably may be considered as a principal factor in determining when seasonal infestation of molluscs occurs, and the probable period of infection of the definitive host as a result of escaping cercariae.

Ross and McKay (6) recorded their first hatch after eight days at 26 to 27.5° C.; at 25° C. the mass hatch took place on the tenth day, and no development took place below 10° C. Mattes (4) stated that eggs develop only on addition of water and above 9° C., development being slow between 9 to 15° C. Development to miracidia, he noted, required two weeks at 20 to 30° C. Shaw and Simms (7) found that ova hatched at varying periods under room conditions, some in as short a time as 14 days; however, some cultures continued to hatch at intervals up to 13 months and 20 days after collection.

From a comparison of results obtained on hatching with those of other investigators, it is apparent that the times of hatching with relation to temperature may show wide variations. At uniformly high temperatures, Ross and McKay (6) obtained a hatch in eight days, with a temperature range of 20 to 30° C.; Mattes (4) found that development to miracidia requires two weeks at 20 to 30° C., 20 to 40 days at 17 to 18° C., the optimum being 20 to 25° C. In the author's observations, ova were seen to hatch in 13 to 15 days, at a temperature of 20° C., the mass hatch usually occurring at about three weeks.

A factor of major importance and interest to be considered, with the severe winter temperatures experienced in Canada, is the effect of low temperatures on ova. Shaw and Simms (7) found that if newly collected ova and well embryonated ova were exposed to a temperature of −12° C. for 24 hr., newly collected ova hatched after thawing, but those containing live embryos at the time of exposure were destroyed. Because of the author's limited supply of ova, it was necessary to keep them in the refrigerator at 2 to 4° C. The ova were placed in the refrigerator in November 1933, and until March 1935 these gave a supply of miracidia when required, hatching usually within three or four weeks after removal to room temperatures or 20° C. After a period of five months, on removal from cold storage to a temperature of 20° C., ova were found to hatch in 16 days. After a period of 11 months, hatching results were still satisfactory, though the percentage hatch appeared to be slightly decreasing. After 16 months, hatching results were still satisfactory, the ova hatching in from 24 to 30 days after removal from the refrigerator.

It does not appear that the cold storage of ova up to 16 months has any material effect on their ability to hatch. However, the vitality and penetration power of the miracidia might be questioned. This point could not be investigated owing to the fact that a proven intermediate host was not available for exposure to attack by these miracidia. Krull (2) found that eggs held

at approximately 2 to 10° C., for a period of two years, six months, and seven days, upon removal to room temperature, developed and hatched readily in 18 days. These miracidia were used for infestation of laboratory-raised *Pseudosuccinea columella*, which were infected en masse, with positive results.

Shaw and Simms (7) have shown that embryonated ova are destroyed on exposure to −12° C. for 24 hr. To observe the effect of cold on embryonated ova, a culture of ova, just commencing to hatch, was placed in the refrigerator at 2 to 4° C. Hatching was checked, and it was found that for some time following, ova could be removed from this to ordinary room temperature and would almost immediately commence to hatch; the miracidia upon hatching appeared quite normal.

After 10 days at 2 to 4° C., on removal to room temperature, some of the ova hatched within a very few minutes, the miracidia appearing normal. After 14, 37 and 168 days, a number of ova hatched immediately upon removal to room temperature, the embryos within the egg at the longest period having the appearance of being slightly shrunken, but still hatching normally. After a period of nine months, ova were removed to room temperature and a good hatch was obtained, but signs of lack of motility and activity of the miracidia were apparent. After 14 months storage, hatching was slowed considerably, the first ova hatching in 35 min. The mass hatch did not occur until one hour after removal from the refrigerator; only a comparatively small percentage hatched, the embryos appearing to have disintegrated to some extent. These miracidia showed considerable lack of motility, the cilia not functioning normally and the organism moving slowly in a rotary manner.

Darkness did not appear to influence the development of ova to any marked degree, though some delay in hatching was evident. It was noted that after three or four weeks, cultures kept in darkness would usually yield miracidia on exposure to light. The keeping of cultures in darkness was consequently a convenient means of obtaining miracidia when desired.

From the work of Ross and McKay (6) there is no indication that the application of copper sulphate in the field, for mollusc control, would in any way effect the hatchability of fluke ova. They exposed ova to such dilutions of copper sulphate in water as had been found lethal to intermediate hosts, *viz.*, 1 : 1,000, 1 : 10,000, 1 : 100,000 and 1 : 1,000,000. The eggs were exposed to these concentrations for 24 hr., washed, incubated, and found to develop satisfactorily. Luhrs (3), however, claims that sea water has a marked lethal effect on ova, destroying them in 16 days, the miracidia being destroyed in 45 sec.

In order to observe the effect on fluke ova of a few chemicals that might be utilized advantageously on certain pastures, a number of cultures was made using solutions of different chemicals in water. These cultures were maintained at room temperature at about 20° C. Ova cultured in 1% and 5% copper sulphate respectively, showed no signs of development. In 5% potassium dichromate the ova collapsed rapidly, and in 1% sulphate of ammonia

development to the embryo stage was observed, a few ova hatching, but perishing on contact with the media. In 1% kainit, the ova developed to an advanced stage, but perished in the shell prior to hatching.

It seems apparent that in order to prevent ova development by the use of desirable chemicals, exposure to a strong solution for a considerable time would be necessary, a measure that would be impracticable in the field.

By frequently changing the water of cultures, the rapidity of development of the ova seems to increase to a certain degree, the influencing factor probably being the presence of available oxygen. Mattes (4) states that foul water inhibits the egg development and may even kill the ova.

On several occasions, stock storage jars of *F. hepatica* ova were found to be infected with a parasitic fungus. While it did not resemble *Catenaria anguillulae*, a Chytridiacean parasite of *F. hepatica* ova described by Butler and Buckley (1), identification was not possible because the fungus could not be induced to fruit. This parasite may possibly have been responsible for a considerable decline in the percentage hatch of ova kept at 2 to 4° C., though this would not seen to be an optimum temperature for its development.

Some Malformations of *F. Hepatica* Ova

During the course of routine examination of *F. hepatica* ova the opportunity of observing malformed eggs was presented. The eggs in question were from a rabbit harbouring 13 adult flukes, the metacercariae of which had been obtained from snails shipped from Oregon.

Taylor (8) refers to the production of malformed eggs by *F. hepatica* and considers it to be a normal occurrence in the early stages of activity of the generative organs of the liver fluke. The ova examined by Taylor were collected from a sheep that had died from sub-acute fascioliasis, and the majority were observed to be smaller than usual, of a darker colour, and bearing a protuberance at one end. The malformed ova observed in this investigation were obtained from a rabbit infested some nine months previously. A considerable variation in egg size was found, tending to range below the usual sizes recorded. Egg shapes were very irregular and many ova were infertile.

The malformation of greatest interest was the appearance of many ova surrounded with what superficially resembled a uniform gelatinous coating, bearing, in a few cases, a distinct spine on one side of the ovum. The average length of the ovum plus the coating was 180μ, the breadth averaging 121μ as compared with the usual measurements of 130 to 145μ long by 70 to 90μ wide. The coating varied in thickness from 20 to 25μ. Proof is lacking that the coating was gelatinous, but on addition of a little formalin, the ovum collapsed and the coating assumed an elastic condition and a coagulated opaque appearance, which might suggest that some substance of a gelatinous nature was present.

The Miracidia

The period of free activity of the miracidia of *F. hepatica* is a comparatively short one, in many cases being only a few hours. The usual time of hatching of ova cultures was consistently observed to be in the late morning. This may possibly have been due to a temperature change or an increase in the intensity of light in the laboratory. Exposure of well-embryonated ova to bright artificial illumination did not influence the rate of hatching and could not be used as a means of inducing liberation of miracidia. Only on two occasions were miracidia observed to emerge backwards from ova at the time of hatching.

When the development of the miracidium is completed it does not necessarily hatch, but may remain for some time within the egg shell. Addition of cold water has been known to cause hatching, but it is not known whether the temperature change or some more complex factor is responsible. Mattes (4) claims that pH is a very important factor in the hatching of ova. He states that increase of acidity from pH 8.0–8.5 to 5.5–6.0, either by use of naturally acid water or by acidifying the culture water with acids, causes hatching in from 10 to 15 min. Greater acidities (pH 3.0 or lower) are ineffective, while in alkaline water (pH 7.5 or higher) the miracidia fail to hatch and eventually die within the shells. Mattes further states that lowering of temperature without increased acidity is ineffective, and that in nature rain is probably the factor that raises the acidity. This causes hatching, owing to the fact that a favourable acidity increases activity of the flame cells and the general activity of the miracidia inside the shell. The actual opening of the operculum is caused by intake of water into the fluid vacuole.

Shaw and Simms (7) found that miracidia would live for 24 hr. after hatching, but the majority died after 8 hr. Ross and McKay (6), having observed that eggs hatching in the spring yielded miracidia that lived longer than those hatching in the summer, carried out experiments to ascertain whether temperature influenced longevity. Miracidia, after hatching, were kept at temperatures varying from 8 to 26° C. Those kept at low temperatures were active for 48 hr., and some individuals remained active for 72 hr. Those at the higher temperatures however, were all dead within 8 hr., temperatures between these extremes providing corresponding periods of longevity.

From the foregoing data, it seems that the probable periods of maximum infection of the molluscan hosts in Canada would be in the spring or fall. Lower water temperatures and resultant increased longevity of miracidia would permit greater possibility of locating the required intermediate host.

During the early part of this investigation, observations were made of the reactions of the miracidia to the molluscs collected from the field. In order to permit detailed observations the larger species were exposed to the miracidia in a Syracuse watch glass, but the smaller snails could be observed conveniently under the binocular microscope in the concavity of a hanging-drop slide. The

desired number of miracidia was admitted by means of a glass pipette, having previously been taken from a culture at the peak of its hatching period.

A further method of exposure was to place a known number of embryonated ova in a glass vial, suspend this vial below the water surface in the aquarium tank by attachment to a cork, and allow the miracidia to hatch and be liberated as they would under natural conditions. This method was found very suitable because the desired number of ova could be used according to the number of snails in the tank, and over-infestation was unlikely to occur. Furthermore, the glass vial could be removed and examined to ascertain how many of the ova had hatched.

In large aquarium tanks when no individual observations were made, miracidia or embryonated ova were admitted in such numbers as would be unlikely to occur under field conditions. These mass infestations were repeated at intervals to permit every opportunity of infection of the molluscs. A further means of exposure employed was to add infected faeces direct to the tank and thus ensure a hatch under as near field conditions as possible.

Behaviour of Miracidia in Presence of Molluscs

During the course of this investigation, 11 species of molluscs from the lower St. Lawrence valley were exposed frequently to miracidia, but none of these was implicated as the intermediate host of *F. hepatica*. Several species did not possess close relationship to the Lymneas, to which the suspicion of vector of this fluke is usually attributed.

To Mr. A. La Rocque of the National Museum of Canada, and Dr. F. C. Baker of Illinois, the author is greatly indebted for the identification of the following species of snails:—

Helisoma infracarinatum (Baker)
H. antrosum (Say) var. *unicarinatum*
Stagnicola palustris elodes (Say)
S. palustris var.
Physa gyrina (Say)
Amnicola sp.
Fossaria obrussa (Say)
F. obrussa exigua
F. umbilicata
Succinea retusa (Lea)
Cochlicopa lubrica (Mull.)

Many representatives of each of these species were exposed individually, kept under observation during exposure, and later killed and examined for any stages of intra-molluscan development. In no instance were any stages of development of *F. hepatica* observed in these snails. Later in the investigation over 25 tanks of snails were exposed to mass infections of miracidia, these infections being repeated at intervals of a few days, thus permitting ample opportunity for infestation of the intermediate host. Abnormal mortality of the molluscs was never noticed after exposure. Those that died were examined immediately, but did not yield any stages of *F. hepatica*.

On exposure to miracidia, all species were readily attacked, those of non-Lymnaea groups being attacked almost as readily as those of the Lymnaea types. Although the attack was deliberate in many cases, it was also acci-

dental in others, for on many occasions miracidia were observed to swim by the snails without attacking them. This occurrence, however, was also noted when a proven vector, *Gyraulus ferruginea* Haldeman, was exposed to their attack. The miracidia would definitely attack, but they would also swim around in close proximity without becoming attracted or attempting attachment to the tissues of the snail. During the periods of exposure, only one miracidium was definitely seen to enter the tissues of the mollusc.

Mattes (5) has shown that there is no evidence of chemotaxis in the finding of the intermediate host by the miracidia. He observed that they appear to reject hard bodies such as stones, but they repeatedly attack soft-bodied animals. He considers that the finding of an intermediary is a random process.

In many cases miracidia, on introduction of a mollusc to their dish, would attach themselves to any exposed portion of it. At times they would detach immediately and at others would remain attached for 10 min., attempting penetration of the host's tissue, but never succeeding. On several occasions, after attacking for some little time, a paralysis of the organism would appear to occur, the miracidium finally dropping away from its point of attachment as if exhausted. It is possible that this paralysis may be the result of some defence mechanism on behalf of the mollusc. However, the snails appeared unaware of any external irritation during periods of attack.

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LACUSTRINE INVESTIGATIONS IN THE GASPÉ PENINSULA[1]

By A. J. M. Honeyman[2]

Abstract

A study of the temperature variations in Ross Lake indicates that it is a typical "second order" lake. The seasonal variations are explicable in terms of the changes in air temperature and the resulting effects on temperature and density of the water. Thermal stratification is clearly indicated. Seasonal variations in the dissolved oxygen content of the water are largely dependent on temperature changes. The variations in acidity, alkalinity, and hydrogen ion concentration are dependent chiefly on temperature changes affecting solubility of carbon dioxide, and on the limy nature of the lake bottom. Analysis of the oxygen distribution indicates a eutrophic condition, but there is as yet insufficient quantitative information about the biological conditions to warrant definite conclusions.

Introduction

In the Gaspé peninsula, about 15 miles from the village of Gaspé, are eight small lakes that are used by the Hatcheries Branch of the Department of Fisheries as natural rearing waters for trout brood stock. The general features of these lakes are discussed in a paper by Taylor and Lindsay (10). The lakes are similar in some respects, but diversified as to size, depth, and type of bottom. They are, with the exception of Grand Etang Lake, within a radius of three miles in a region sufficiently mountainous and inaccessible to be visited only by local woodsmen. They vary in size from Fourth Lake, with a length of about 1.6 km. and a breadth of 800 m., to the pond-like Trail Lake, whose length and breadth each approximate 180 m. The depth varies from slightly over 30 m. in Grand Etang to between 3 and 4 m. in Trail Lake. The entire bottoms of the smaller lakes and parts of those in the larger ones are formed of limestone or a deposit of soft, almost pure calcium carbonate. There is very little of this deposit in Fourth Lake. The importance of such deposits in the economy of lake productivity is worthy of investigation. These lakes afford an opportunity to investigate problems of fertility and productivity, which may be of general application.

For the past three years an intensive study has been made of Ross Lake with reference to topography, water temperatures, dissolved oxygen content, acidity, alkalinity, and hydrogen ion concentration. Similar but less extensive studies have been made on several other lakes. A study of biological factors, including the use of algal photosynthesis as a measure of productivity, is now being undertaken.

Methods

Temperature

Temperatures were obtained with a Richter and Weise reversing thermometer at one point within the 21-m. contour. In winter they were taken through a hole in the ice, in summer, from a boat.

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Dissolved Oxygen

The method used in the determination of dissolved oxygen is a modification of the standard Winkler technique, the chemicals used and the reactions involved being the same. This method, described by Nicloux (8), was found very suitable to the conditions of our work. The advantages of the technique are that smaller samples of water are required and that no chemicals need be added at the time of collection. Only about 25 ml. of water is used for an analysis. The analysis itself is very rapidly carried out, owing to the fact that small quantities of chemicals are used. Samples analyzed at the time of collection and others that had been kept in bottles under water and packed in ice, gave identical results. The water samples were collected with a Richter and Weise reversing water bottle and run into glass-stoppered bottles that were stored in special ice-cooled metal containers. The analyses were made in a laboratory on shore.

Acidity and Alkalinity

Free carbon dioxide content and alkalinity were determined by neutralization of the sample in a Nessler tube with standard sodium hydroxide and sulphuric acid solutions respectively, phenolphthalein being the indicator. The technique is that of the American Public Health Association (1, p. 32 *et seq.*). The carbon dioxide determinations are expressed as parts per million of carbon dioxide and the alkalinity as parts per million of calcium carbonate.

Hydrogen Ion Concentration

These determinations were made colorimetrically by use of the indicators brom thymol blue, phenol red, chlorphenol red, and metacresol purple.

Ross Lake Topography

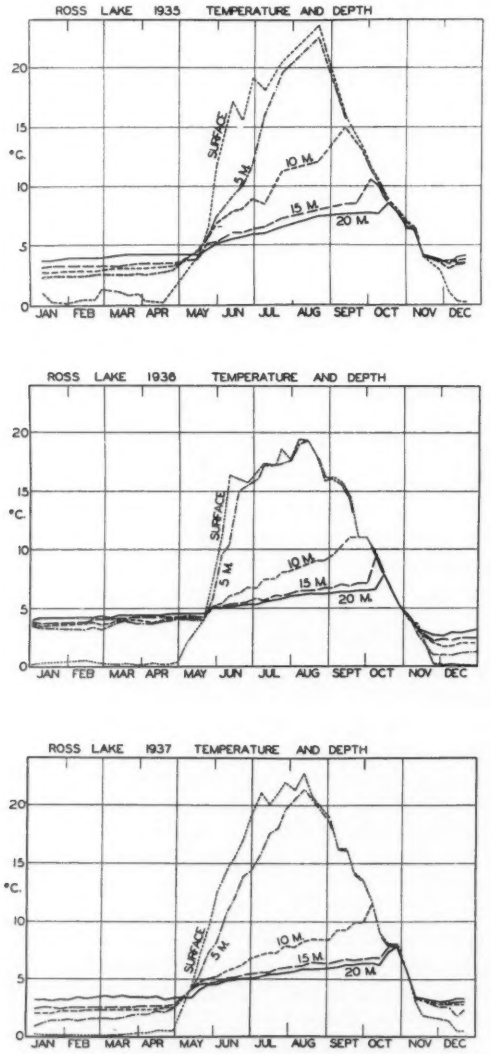
Ross Lake is situated in the county of Gaspé South, township of Baillargeon, near the eastern extremity of the Gaspé peninsula and about 15 miles from the village of Gaspé. It is on top of a high ridge, with the surface at an altitude of 164 m., a situation that provides a comparatively small drainage basin and no inlet brooks. Its outlet is small and empties into the St. John River. The drainage area is only about three times the area of the lake itself, which is fairly regular in shape and measures 710 m. in length and 610 m. in breadth. Depth contours have been mapped and were derived from an extensive series of soundings taken at 100-ft. intervals over the surface when it was covered with ice.

There are two deep areas in the lake, one of 23 m. and another of 20 m. A very large percentage of the lake is over 9 m. in depth. The only shallow areas of any extent are in the vicinity of the discharge. More than half the drainage area is covered by a poor growth of young poplar.

Temperature Conditions

The temperature data on conditions in this lake are plotted over yearly periods in Figs. 1, 2, and 3. These three graphs show the seasonal variations

in temperature for the complete years 1935, 1936, and 1937, the measurements being made at weekly intervals, and at depths of 20, 15, 10, and 5 m. and the surface. Examination and comparison of these graphs disclose some interest-



FIGS. 1 – 3. *Curves for Ross Lake, 1935 (top), 1936 (centre), and 1937 (bottom), showing the seasonal variation of temperature with depth. Readings taken at weekly intervals all from one station.*

ing facts. Their similarities may be summarized as follows. The spring overturn occurs about May 15, and the period of complete mixing is confined to about a week. Then thermal stratification sets in and becomes most pronounced in mid-August, when a difference as great as 8° C. exists between the water at 5 and 10 m. The autumn overturn occurs about October 15; but the stratification that follows is evidently a slower process, as indicated by the curves for different levels. The temperatures do not begin to diverge for about a month after all levels reach the same temperature. This is to be expected, because once the ice cover is present conditions are relatively static, and the stratification is brought about only by the effect of gravity. When the winter stratification is complete, the temperature varies in a regular manner from 0.1° C., at the surface (*i.e.*, the point just below the ice) to approximately 4° C., the temperature of maximum density, at the bottom. Evidently when disturbing external forces are excluded by the ice the water layers are distributed according to their density. During the ice-free months the temperature—depth relation is exactly reversed, though the density relation holds, the layers of water farthest removed from the temperature of maximum density being nearest the surface.

Graphs for all years show also that the maximum temperature for any depth is reached later in the season as the depth increases. This is to be expected since the only source of heat for the lake is the warm air above it, and the upper layers of water must be heated before those below can be affected. This is a slow process and is unlikely to be aided to any extent by currents, due to the resistance of the stratified water. The October circulation period is made possible by the disappearance at this time of thermal stratification, and it is shown that this homothermous condition is brought about not by any considerable warming of the lower water levels but almost entirely by the cooling of the surface layers to a temperature equal to that of the bottom layers.

No precise study of the thermocline can be made from the data, since the depth intervals (5 m.) are too great, but there are certain points of interest. In 1935 one definite thermocline emerges in July, at a depth between 5 and 10 m. By September its position has sunk to between 10 and 15 m. In 1936 on the other hand, the thermocline remains established between 5 and 10 m. from June until early September. In 1937 the typical depression of the thermocline with the advancing season is shown more clearly than in 1936.

As would be expected, the maximum temperature of the epilimnion shows some variation from year to year, but the time is always between August 7 and 21.

Some observations will now be made on the intermediate levels of 5, 10, and 15 m. In 1936, May 21 is the only date (as judged from temperatures) when there is practically complete mixing of the water of the lake. This is the focal point of the spring overturn. The temperatures at 20, 15, 10, and 5 m. and at the surface on this date are respectively 4.5, 4.2, 4.1, 4.1, and

4.0° C. On May 17, the previous date on which readings were taken, the surface water was at 2° C. while on May 28, it had warmed to 7.4° C. The fall overturn is a slower process than the spring circulation, and the whole body of water in the lake remains homothermous from October 15 to November 12, though during this interval it cools gradually from 8° to 3° C. A few days after November 12 the ice cover appears, and a rapid cooling of the surface water to its winter level takes place.

Measurements from these intermediate levels show also the gradually changing seasonal heat distribution in the lake. At the beginning of June 1936 the water begins to warm from the surface downward, the epilimnionic region reaching a depth of 10 m. on September 10. This region maintains a temperature of 10° C. or higher until October 1, when further cooling and mixing equalizes the temperature of the whole body of the lake. These observations apply also to the data obtained in 1937.

The lake may then be classified on the basis of the above temperature relations, as one of the "second order" in the scheme developed by Forel and Whipple.

Dissolved Oxygen

Measurements of dissolved oxygen in Ross Lake are available for the complete years 1936 and 1937. The data have been calculated in parts per million and percentage saturation. The results for the surface and 20 m. depth are given in Figs. 4 to 7.

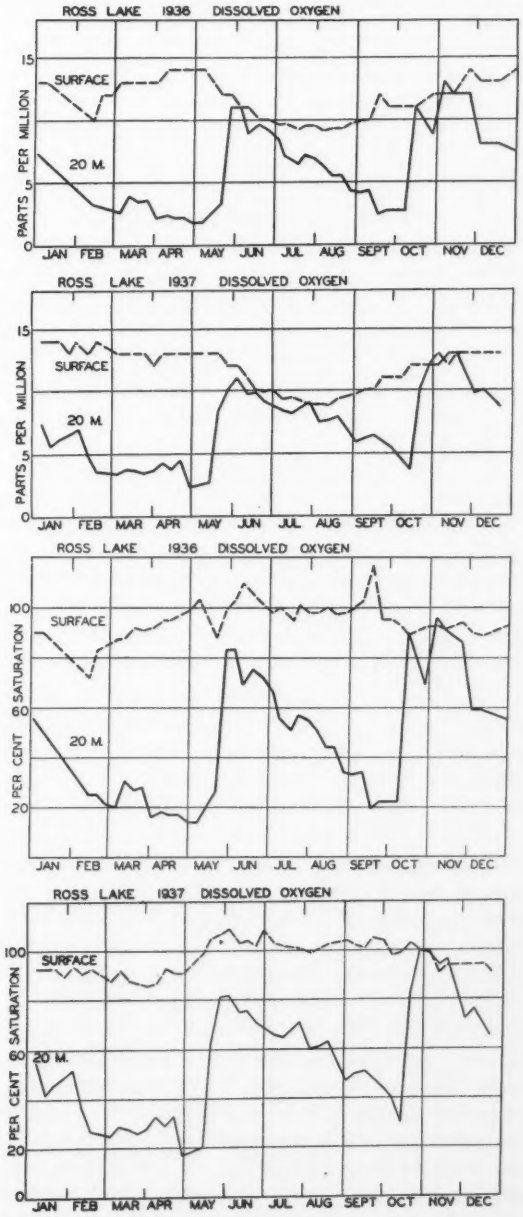
Examination of the graph showing the seasonal variation in absolute dissolved oxygen content for 1936 (Fig. 4) reveals the following significant points.

The oxygen content of the surface water is high (12 to 14 p.p.m.) when the ice cover is present, that is, from January to May and from October to December inclusive. This is due chiefly to the fact that the temperature is approximately 0° C., at which point the solubility of the gas is at a maximum.

The drop in oxygen content of the surface water from June to August is due to the higher water temperature and consequent slighter solubility of the gas. The initial drop results from the water in the lake being mixed at the spring overturn, the surface water losing oxygen to the lower levels.

The same graph shows how the oxygen content of the whole lake becomes uniform within the short period of a week at the end of May as a result of the spring overturn, though this process probably does not greatly increase the absolute quantity of oxygen in the lake. The rapidity with which this uniformity develops is a result of the rapid disappearance of the ice after it begins to melt. Once it has broken, the ice disappears within a day, and the mixing process begins immediately.

Another point shown by the data is that in July and August the oxygen content is not greatest at the surface, as one might expect, the maximum being at 10 m. It is supposed that the higher temperature of the surface water at this time reduces solubility, in spite of the fact that more oxygen is available



FIGS. 4 – 7. FIGS. 4 AND 5 (TOP). *Ross Lake 1936 and 1937. Seasonal variation in dissolved oxygen content shown for surface and 20 m. depth. Measurements all made at same station.* FIGS. 6 AND 7 (BOTTOM). *Ross Lake 1936 and 1937. Dissolved oxygen expressed as a percentage of the maximum amount soluble in distilled water at the same temperature.*

at the surface than at 10 m. For the same period it is seen that the decline in oxygen content above 10 m. involves no decline in percentage saturation. It would be desirable to study the presence in these regions of algal growths, as a probable factor in this situation.

Water at the 20 m. level after June 1 gradually loses oxygen (a) because it is too far from the source of supply to receive any by diffusion, and being below the thermocline it is unlikely that water currents will supply it to any extent, (b) because of its slowly rising temperature and (c) probably because of decomposition activity, though the extent of such processes cannot yet be estimated in this lake.

At the autumn overturn the oxygen content again becomes uniform throughout. The drop in oxygen content of the lower levels with the advent of the ice cover in November is due to diffusion of gas to the colder surface layers.

A curve relating percentage saturation of oxygen and depth for 1936 is given in Fig. 6. The data have been calculated from the measurements of C. J. J. Fox, as tabulated by G. C. and M. C. Whipple. It is noted that this temperature adjustment has practically no effect on the shape of the 20 m. curve, its outline being the same as that for absolute oxygen content at the same level. A noticeable change has been produced in the surface water curve. The decrease in oxygen content during the summer months is now not shown because, as was noted above, this decrease was due to smaller absolute solubility of the gas in the warmer water. The percentage of oxygen dissolved is only slightly higher during the summer season than during the winter, the difference being due probably to photosynthetic activity.

In regard to the lower and upper limits of oxygen concentration the following points may be noted. The lowest concentrations are found in bottom water during the stagnation periods and vary from 2 to 4 p.p.m., the lowest value obtained in the two years being 1.8 p.p.m. The highest values are found in surface waters during the winter months and frequently reach 14 p.p.m.

Values for percentage saturation are as low as 13.7 at a depth of 20 m. in March and April before the ice melts. Readings for surface water of over 100% saturation are frequently found, though no large masses of algae or other aquatic plants are present in this lake. Comparison of the oxygen curves for 1937 with those of 1936 indicates a remarkable similarity. The spring and fall overturns each come a few days later in 1937. The percentage saturation curves for 1937 (Fig. 7) are likewise similar to those of 1936.

In regard to the oxygen content at depths intermediate between 20 m. and the surface, from January to June, 1936, the amount of oxygen decreases in a regular manner from surface to bottom, though after the spring circulation there is a considerable increase at the lower levels. At the overturn the whole body of water is almost saturated with oxygen. In July, however, a different situation develops, and, when the temperature of the epilimnion rises, the amount of gas dissolved in it decreases to about 9.5 p.p.m. The intermediate

region (about 10 m.) has the greatest concentration of oxygen at this time. This situation does not last long, however, for by September the epilimnion has extended downward to include the 10 m. level. In November the autumn circulation brings about an even, vertical distribution of oxygen. The data for 1937 confirm these observations.

Further Analysis of Physical and Chemical Conditions

A summary of the chief physical and chemical characteristics of Ross Lake is found in Table I. The small area and volume make its comparison with other lakes on an equal basis difficult. The volume of the hydrographic epilimnion (0 to 10 m.) is about three times that of the hypolimnion. Such a ratio is considered to be an indication of a eutrophic condition. The annual heat budget, calculated by the method of Birge, is lower than that usually found, probably because of the small volume and area.

TABLE I

ROSS LAKE. SUMMARY OF PHYSICAL AND CHEMICAL CONDITIONS

Date	Aug. 6, 1936		
Latitude	49		
Altitude (m.)	164		
Area (km.^{2})	0.26		
Length (km.)	0.71		
Breadth (km.)	0.61		
Mean depth (m.)	9.1		
Max. depth (m.)	23		
Volume (m.^{3})	2394×10^3		
Vol. E. (0–10 m.)	1776×10^3		
Vol. H. (10 m.–bottom)	618×10^3		
Vol. H./Vol. E.	0.35		
Annual heat budget (gm. cal./cm.^{2})	11,100		
		Oxygen amounts	
		O_2E. (cc./l.)	6.8
		Total O_2E. (cc.)	1208×10^7
		O_2H. (cc./l.)	5.2
		Total O_2 H. (cc.)	321×10^7
		$O_2H./O_2E.$	0.27
		Oxygen deficits	
		ΔE (cc./l.)	0.2
		ΔH (cc./l.)	3.5
		$\Delta H + E$ (cc./l.)	1.05
		Oxygen capacity	
		O_2A (cc./cm.^{2})	0.97

An analysis of the oxygen conditions has been made by the methods of Thienemann (11). The calculations were made for Aug. 6, 1936, a date representative of midsummer conditions. The ratio O_2H/O_2E of 0.27 is typical of that found in eutrophic lakes (9). In calculating the oxygen deficits the oxygen solubility values used are those of Whipple and Whipple. The deficit for the epilimnion (ΔE) is slight, 0.2 cc./l. indicating that this layer is practically saturated. In fact, on some days negative deficits are found, indicating supersaturation in this layer. For the hypolimnion the oxygen deficit is considerably greater, amounting to 3.5, though not high in comparison with many of the eutrophic lakes listed by Rawson. The deficit for the whole lake, 1.05, is derived from ΔE and ΔH by considering the respective volumes of E and H, and as a result is closer to the ΔE value. These statements may be confirmed in a general way by examining the saturation curves (Figs. 6 and 7). The analysis of oxygen conditions indicates a

eutrophic condition, but more definite conclusions cannot be drawn until further biological and chemical information is available.

Acidity and Alkalinity

The data with reference to these factors are plotted on the same graphs (Fig. 8 for 1936 and Fig. 9 for 1937), the carbon dioxide being represented above the zero line and the alkalinity figures below it. Only data for measurements at the surface and 20 m. depth are shown on the graph.

At a depth of 20 m. the carbon dioxide content of the water increases gradually from January to the middle of May, when the spring overturn takes place. It then drops to almost zero, begins to rise during the summer, and falls again with the autumn overturn.

The surface water shows less variation throughout the year. It is acid from December to May and on the alkaline side during the summer months. The conditions are similar in 1936 and 1937. It may be said that the surface waters of Ross Lake contain little or no free carbon dioxide. This may be explained by the fact that the water is constantly agitated and the dissolved carbon dioxide escapes by diffusion into the air, and by the buffering effect of the carbonates present.

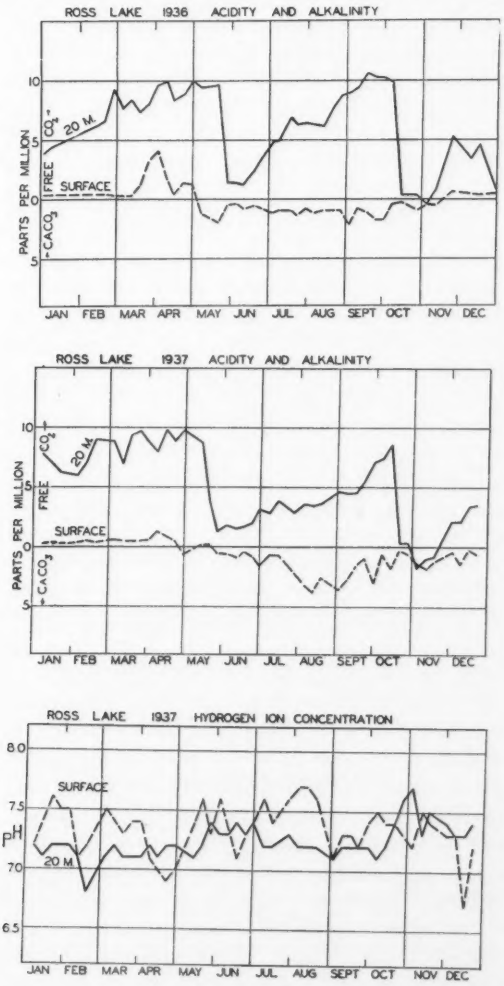
From January to April 1936, the carbon dioxide content of the lake increases gradually from surface to bottom. In May the surface and 5 m. levels lose all their carbon dioxide and become alkaline. This condition is maintained until September, when the 15 m. level also becomes alkaline. For a brief period at the autumn overturn (November 5) the whole body of water is alkaline in reaction.

It is noteworthy that at two periods of the year the lake loses its entire content of carbon dioxide, which is essential for organic productivity. In addition to this reduction at the circulation period, the activities of certain marl-forming algae (Chara) and pondweeds (Potamogeton) precipitate carbon dioxide as insoluble carbonate. Any photosynthetic activity has the same effect, but these particular genera are found in great quantities in Ross Lake; in fact, they compose almost the entire rooted standing plant growth. Their activity is indicated by the large beds of marl on the bottom of the lake. These marl deposits are formed chiefly in the epilimnionic region. In some other lakes of the vicinity, shallow enough to be homothermous at all times, the deposits cover the entire bottoms. This distribution is in accord with the observations of Kindle (6), who points out that all the factors tending to produce marl deposits are most active in the warmer surface layers of the lakes.

Hydrogen Ion Concentration

Data on pH are available for the period August to December 1936 and the year 1937, and the results of the surface and 20 m. depth determinations of the latter year are plotted in Fig. 10. The maximum pH range in Ross Lake is from 6.8 to 8.1.

A comparison of the free carbon dioxide graph for 1937 (Fig. 9) and the pH graph for 1937 (Fig. 10) shows that in spite of great irregularity in individual measurements, there is a considerable correspondence between the seasonal changes in the two cases. For instance, a fall in carbon dioxide content at 20 m. in mid-May corresponds to a rise (more alkaline) in pH at that time.



FIGS. 8 – 10. FIGS. 8 AND 9 (TOP AND CENTRE). *Ross Lake 1936 and 1937. Free carbon dioxide indicating acidity is shown in the upper part of the graph while the lower part shows alkalinity expressed in parts per million of calcium carbonate.* FIG 10 (BOTTOM). *Ross Lake 1937. Showing the seasonal variation in pH at the surface and 20 m. depth.*

Increasing alkalinity of the surface water in July and August corresponds to an increase in pH at this time. Similar relations are observable at other seasons.

On the whole the seasonal variations in pH are slight. It is suggested that this may be due to the buffer action of the monocarbonates present. As mentioned above, large quantities of calcium carbonate are present both as bottom deposits and as constituents of, and incrustations on, water plants such as Chara and various Potamogetons. Any increase of free carbon dioxide or carbonic acid content in the presence of calcium carbonate is immediately neutralized, and little increase in pH occurs. Likewise, removal of carbon dioxide by photosynthetic activity or by agitation of the water results in the decomposition of bicarbonate into carbonate and carbonic acid, thus increasing the acidity.

Summary of Physical Conditions

The physical conditions in Ross Lake are in most respects favourable to aquatic life. In comparison with the size of the lake, there is a large body of deep water, a considerable part of which is cold owing to the presence of a definite thermocline. However, the large proportion of deep water results in a corresponding lack of shallow areas adequate for organic growth. Another marked deficiency is in the absence of inlets to the lake, because of its position on a height of land. The drainage basin itself is covered by a scanty growth of young poplar and there is very little top soil over the rocks. The temperature, typical of such lakes, is favourable to fish life.

The dissolved oxygen content shows the expected seasonal variations. Though authorities differ on the minimum and optimum oxygen requirements of fish, it is unlikely that the supply here becomes inadequate except at the deep levels during the winter months. However, a situation unfavourable to fish life may exist in the coincidence of low oxygen periods and time of acid pH.

The lack of carbon dioxide at certain seasons is shown by the data and curves. The pH variations follow in general the carbon dioxide cycle, but the seasonal changes are less well defined and in any case are confined to a range of pH 6.8 to 8.1.

Biological Conditions

In 1887, Forbes (5) spoke of the lake as a closed community comparatively slightly affected by events outside it, and in 1935 Welch (12) stated that "in a lake with a small inflow and outflow of water, relatively small slow additions to the food supply are made from the outside." Klugh (7) and other writers attached importance to the quantity of rooted aquatic vegetation as an index of productivity of lakes. These viewpoints have special application to the lakes herein studied. Plant life in all these lakes is scarce. The only plant that can be described as abundant and well established is *Chara*. The rest of the plants are seen as scattered specimens that have made little progress in occupying the large vacant areas of lake bottom. There are two

possible explanations of such a paucity of plant life. That of the absence of shallow areas near shore and the regularity of the shore line applies to Grand Etang, McLaren, and Ross Lakes. The other and related explanation, that of the absence of sufficient food material to support any considerable plant population, is generally applicable. The lakes are practically devoid of soil bottom, the bottom consisting of limestone or of soft marl deposit often a foot or more thick. Certain conditions tend to restrict the bottom deposits to this marl. It is strangely characteristic of these lakes that they are situated on high elevations and consequently possess comparatively small drainage basins. This reduces the run-off of organic material into the water. In any case, the forest cover about Ross and Fourth Lakes is very light, and the soil only slightly covers the rocky ground. Also, owing to the small size of the lakes the effect of wind and wave action in eroding the shores is slight. It should be noted as well that in common with all other lakes at this latitude, organic activity on the land surrounding the water is in an even more static condition than in the water itself for more than five months of the year. The apparent inability of these lakes to support a large fish population for more than a few years is an indirect indication of the deficiency of available organic material.

The following genera of water plants are generally though sparsely distributed throughout the lakes: Chara, Potamogeton, Myriophyllum, Nymphaea, Sparganium, Eriocaulon, Fontinalis, Equisetum, Utricularia, and Oedogonium. Of these, the lime-consuming plant Chara and the Potamogetons, on which quantities of lime are deposited, are the dominant species.

A more quantitative study of the biological factors will be undertaken before more definite estimation of their effects is made.

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